PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
Volume 21 February, 15, 1935 Number 2

INERT REGIONS OF CHROMOSOMES AS THE TEMPORARY PRODUCTS OF INDIVIDUAL GENES

By H. J. Muller and S. M. Gershenson

Institute of Genetics, Academy of Sciences, Moscow

Communicated January 8, 1935

Soon after the phenomenon of no crossing-over in the male was discovered, it was pointed out (Muller 1914, 1918), that this circumstance must lead to the accumulation of recessive mutations, and even losses of some genes and duplications of others, in any chromosome which, like the *Y*, is constantly carried in heterozygous condition in a sex in which no crossing-over occurs. The lack of active genes in the *Y* was thus interpreted. It was then taken for granted, however, that the visible material of the *Y* chromosome was of a nature fundamentally similar to that composing the bulk of other chromosomes, and consisted of more or less inactivated genes together with the usual amount of accessory chromatin and other substances that may normally accompany genes in the chromosome as seen at metaphase. When later it was shown (Muller and Painter, 1932), that the *X* chromosome had a similarly inert region, homologous to that of a part of the *Y*, it was concluded that the former had probably been derived from the latter by a kind of translocation, and it was naturally assumed that the inert region of the *X*, like that of the *Y*, consisted of inactivated or "degenerated" chromatin.

At this later date, it was, to be sure, realized that the chromosome material of both the *Y* and *XI* (the inert region of the *X*), might really be relatively devoid of genes, rather than full of degenerated genes, and that the actual gene-string in the inert regions might therefore be much smaller, in proportion to the bulk of visible chromatin, than it was in the "active" regions of the chromatin. But it was found by Oliver (1932) that, in the case of inversions, breaks probably occur in the inert region, as compared with the active region of the *X*, in somewhat the same ratio as that which the bulk of the inert region as seen at metaphase bears to the bulk of the active region (further evidence of this sort will be referred to below), and this was taken by Muller and Oliver as an indication that the inert and active regions were probably constituted in a similar way, an inert region

thus containing a chromonema of similar length, degree of coiling and breakability to that in an equal amount of an active region (see Oliver, *Ibid.*). The alternative to this would have been the assumption, which at that time was less plausible, that, length for length of chromonema, breakage occurred more readily in the inactive region, in which the chromonema was shorter (i.e., coiled to a lesser degree).

A review of the evidence now at hand from various sources, however, makes the conclusion now much more probable that the inert regions of the *X* and *Y* are fundamentally different in structure from the active regions and do not consist of a row of degenerated genes arranged single file in a coiled chromonema like the functional genes in the active region, but that they consist essentially of non-genic material derived from a very few specific active genes, between which breakage takes place much more readily than between genes in the chromonema of the so-called active region. Whether these chromatin-producing genes of the "inert region" lie in the form of a coiled chromonema in their region of the chromosome at the time of mitosis remains a question that it is still difficult to decide.

Evidence for the above conclusion is to be found in several different directions. Most telling is the fact that in various cases of breakage within the inert region of the *X* or *Y* chromosomes, certain large sections of specific size seem to be broken off or carried over *en bloc*. Thus in all of Stern's and of Kaufmann's cases of exchange between the *X* and the *Y*, when an arm of the *Y* was broken off or transferred either the whole of the arm or just half of the long arm was concerned. Again, in the deletions of the *X* chromosome examined by Painter and Muller (1929; also Muller and Painter, 1929) the size of the piece of the *X* remaining was sensibly the same in the different cases in which the locus of bobbed was present—amounting to approximately one-third the length of the chromosome, as we would expect if exactly all of the inert region were present, in addition to a negligible section of active region from the left end. In another case (deletion 24, described by Muller and Painter, 1932) in which the bobbed locus was not present, the fragment was much smaller, being only a few times the size of the fourth chromosome. This fragment must have consisted mostly of inert material, since only that part of the active region to the left of scute was present here, and this inert material must be that which is associated with the fibre attachment locus or other unrecognized loci close to the latter. Conversely, in the case of bobbed-deficiency studied by Dobzhansky (1932, exhibited to the Sixth International Congress of Genetics), the deleted chromosome lacked almost exactly that amount of material which was present in the deleted *X* chromosomes of Painter and Muller, namely, the inactive material, and it contained the amount of material which was absent in the previously described deletions, namely, the active material. This then constitutes evidence from the reverse di-

rection for the same conclusion, namely, that the inert material is in definite blocks of comparatively large size. The chromosome described by Gershenson (1933), in which by crossing-over between two different inverted chromosomes (scute^4 and scute^8, respectively) a deficiency for the region of bobbed was produced, likewise lacks a region nearly equal to the length of the inert region itself, i.e., Gershenson's crossover chromosome and Dobzhansky's deficient chromosome seem to be of sensibly the same length. All this would lead us to the conclusion that, where the bobbed locus was present, nearly all of the inert region was also present, with the exception of a small section—perhaps one-fourth of the total—which is associated with the spindle fibre locus. In the *Y* chromosome the bobbed region is apparently of similar length to that in the *X*, constituting about one-half of the long arm.

Studies of Panshin and Muller (unpublished) on translocations between the *Y* chromosome and the fourth, furnish another, though less direct, line of evidence in the same direction. They find that in the great majority of translocations of this type—at least 90 per cent—one or more of the genes of the *Y* chromosome necessary for the fertility of the male, have become so affected by their altered position as to be no longer capable of producing the effects necessary for fertility. This indicates that these fertility genes are in all these cases located near the point of breakage. Since, however, the *Y* chromosome at metaphase appears rather long, and since there are probably not many fertility genes, the result is hard to understand, unless we suppose that in the ordinary "resting" stages of functioning cells, these genes lie close together and are not separated by the great bulk of inert material seen at metaphase.

In view of this interpretation, the observation of Painter (1933, 1934a and b) that the inert regions of the *X* chromosome are hardly, if at all, to be found in the chromosome structure of the cells in the salivary glands, becomes understandable, and in fact constitutes further evidence for our interpretation itself. We may suppose that in these stages when the genes are functioning in cell metabolism, they do not produce the great local accretions of material to be observed at metaphase in the inert regions, and that the salivary gland chromosomes, therefore, give a truer picture of the chromonema, in so far as the latter represents a string of real genes in which the different genes occupy comparable lengths of the thread. Whether or not the inert material that was present at metaphase still exists somehow in the resting nucleus, and, if so, what its functions may be, is another question. Its functions in segregation and crossing-over likewise remain important subjects for study.

While the above conception disagrees with the idea of the inert region as representing a degenerated gene string, it does not contradict the notion that a process of degeneration and of loss did occur in the past evolution

of the *Y* chromosome, owing to the protection from natural selection afforded by its continually heterozygous condition. The loss, however, has probably, by this time, become complete with regard to all genes that are no longer needed, and the material that is seen, instead of consisting of "degenerated" genes and the material accessory to them, existing in the proportions usual for genes and their accessory material, probably consists almost entirely of the accessory material—i.e., of some sort of especially bulky reaction-product—of the few functional genes that still remain. As for the existence of translocations, to account for the continued similarity between the *Y* and the inert region of the *X*, the reality of the occurrence of such translocations (including "crossovers"), even today, is attested to by findings of Stern and of Kaufmann, previously alluded to.

Attention may at this point be recalled to the fact that, on the view here presented, the finding of Oliver of the occurrence of more inversions affecting the right-hand portion of the *X* than the left-hand portion, is to be interpreted on the basis that there is a much greater likelihood of breakage between two genes which are connected with the inert region than between any two successive genes of the active region. There are several other series of findings which, on our present conception of the inert region, would lead to the same conclusion. One is the fact that x-rays produce translocations of the *Y* chromosome with approximately the same frequency as those of the *X* (Muller and Altenburg, 1930), despite the great dearth of active genes in the *Y* as compared to the *X*. Secondly, there is the fact (Painter and Muller, 1929, confirmed by later work) that the majority of deleted *X* chromosomes obtained by crossing irradiated males to yellow females with attached *X*'s have their right-hand break to the right of carnation (and hence probably in the inactive region) despite the fact that there are many more genes to the left of carnation, which are nevertheless so situated that breaks between them would result in deleted *X*'s having a high viability in this cross. Thirdly, of a series of ten gross (i.e., not minute) inversions studied by the present authors, which involved position effects in specific loci near the left end of the *X* chromosome (yellow, scute, white), six have been found to have their right-hand break in the inert region, and only four to have it in any other part of the chomosome. Fourthly, translocations involving the *X* have their break in the inert region in a large proportion of cases (Patterson, Stone, Bedichek and Suche, 1934).

The excess of breaks in the inert region is in part due to the fact that breaks are more apt to occur near the ends and near the attachment points of chromosomes, as the latter authors show, but this can be by no means the entire explanation. Thus, in work of Levit and of Muller (unpublished), in which the scute 8 chromosome, which has bobbed and most of the inert region located near but not at the left end (and far from the attachment

point), was irradiated, it was found that most of the deleted X's formed had their left-hand breaks in the inert region, rather than in the active region to the left of the latter and still nearer the end, although there are probably many more genes in this left terminal active region than in the inert region. Apparently "simple breaks" of the scute 8 chromosome also occur preferentially in the inert region, as shown by the same authors and also by Patterson (1933). The latter breaks occur about five times as often beyond bobbed as between carnation and bobbed, but among deletions and inversions of X's that were normal before irradiation there seem to be considerably fewer breaks beyond bobbed (between bobbed and the fibre attachment) than between carnation and bobbed, even though the latter interval is further from the end of the chromosome. The breakability, therefore, is in part a specific property of the region concerned, and it is extremely high between genes that lie in the inert region.

On the new conception of the inert region here proposed, various experimental facts require a different interpretation than they would otherwise receive. One example may suffice here. Four different apparently "simple breaks" were produced by Muller and Koerner (unpublished) in the scute 8 chromosome, in the interval between the gene achaete, in the left-hand active region, and bobbed, the left-hand fragment being lost. On the view that the inert region, like the active region, consists of a large number of small, genetically distinct segments (corresponding to genes), the different "truncated" scute 8 chromosomes thus resulting should be of different lengths, lacking not only the left-hand active region (from the left terminus to achaete, inclusive), but also a part of the inert region lying beyond bobbed. They were accordingly tested with the object of determining whether the latter loss were capable of producing any detectable phaenotypic effect. A really adequate test of this kind had not hitherto been possible, since hitherto only individuals deficient for the region including bobbed had been tested, and these necessarily required a Y chromosome to cover their bobbed-deficiency; this Y then might at the same time have covered the deficiency of other genes of the inert region. In the present case crosses were carried out, the genetic details of which it will not be necessary to describe, to allow the production of zygotes homozygous for the truncated X chromosome, but not containing a Y chromosome, and having their deficiency of the active region at the left end compensated for by the presence of a chromosome fragment (the left part of the X of "mottled 5") containing those genes but not containing any part of the inert region of the X (since this fragment of the X is here attached to the right portion of chromosome IV). The results showed clearly that in all cases such females were viable, and the conclusion seemed to follow that the loss of none of the genes in the inert region that normally lay between bobbed and the fibre attachment caused any detectable phaenotypic effect,

even in the absence of *Y*. Now, however, we see that the result probably means only that in all these cases the break was at, or very nearly at, the same point—between bobbed and achaete—and that there was no part of the inert region absent. That part which may be connected with the bobbed locus is necessary for life, in so far as the bobbed gene itself is necessary for life. Whether the genes of the inert region that affect variegation and fertility and possibly other characters, are at other loci, and also have a part of the inert region connected with them, is as yet not definitely known, although the evidence above would indicate that most of the inert region depends on very few genes. Neither do we know whether the fibre-attachment gene of the *X* is in any way specific, or may be completely replaced by a fibre-attachment gene from another chromosome.

As most of the evidence for our present interpretation of the inert region depends upon the small series of cases which were observed some time ago without the present question being in view, and as such comparison is subject to considerable error in the estimate of the length of the pieces of chromosome involved, Gershenson is now undertaking a special study and comparison of a more extended series of cases of this sort. This should give a definitive answer to the question of whether or not the inert region is composed of blocks, and of the number and size of such blocks, and the genes with which they are associated.

Gershenson, S., "Studies on the Genetically Inert Region of the X-Chromosome of Drosophila. I. Behavior of an X-Chromosome Deficient for a Part of Its Inert Region," *Jour. Genetics*, **28,** 297–313 (1933).

Kaufmann, B. P., "Interchange between X- and Y-Chromosomes in Attached *X* Females of Drosophila melanogaster," *Proc. Nat. Acad. Sci.*, **19,** 830–838 (1933).

Muller, H. J., "A Gene for the Fourth Chromosome of Drosophila," *J. Exp. Zoöl.*, **17,** 325–336 (1914).

Muller, H. J., "Genetic Variability, Twin Hybrids and Constant Hybrids, in a Case of Balanced Lethal Factors," *Genetics*, **3,** 422–499 (1918).

Muller, H. J., and Altenburg, E., "The Frequency of Translocations Produced by X-rays in Drosophila," *Genetics*, **15,** 283–311 (1930).

Muller, H. J., and Painter, T. S., "The Cytological Expression of Changes in Gene Alignment Produced by X-rays in Drosophila," *Amer. Nat.*, **63,** 193–200 (1929).

Muller, H. J., and Painter, T. S., "The Differentiation of the Sex Chromosomes of Drosophila into Genetically Active and Inert Regions," *Zeit. ind. Abst. Vererb.*, **62,** 316–365 (1932).

Oliver, C. P., "An Analysis of the Effect of Varying the Duration of X-ray Treatment upon the Frequency of Mutations," *Zeit. ind. Abst. Vererb.*, **61,** 447–488 (1932).

Painter, T. S., "A New Method for the Study of Chromosome Rearrangements and the Plotting of Chromosome Maps," *Science*, **78,** 585–586 (1933).

Painter, T. S., "A New Method for the Study of Chromosome Aberrations and the Plotting of Chromosome Maps in Drosophila melanogaster," *Genetics*, **19,** 175–188 (1934a).

Painter, T. S., "The Morphology of the X-Chromosome in Salivary Glands of Drosophila melanogaster and a New Type of Chromosome Map for This Element," *Genetics*, **19,** 448–469 (1934b).

Painter, T. S., and Muller, H. J., "Parallel Cytology and Genetics of Induced Translocations and Deletions in Drosophila," *Jour. Hered.*, **20**, 287–298 (1929).

Patterson, J. T., "The Mechanism of Mosaic Formation in Drosophila," *Genetics*, **18**, 32–52 (1933).

Patterson, J. T., Stone, W., Bedichek, S., and Suche, M., "The Production of Translocations in Drosophila," *Amer. Nat.*, **68**, 359–369 (1934).

Stern, C., "Untersuchungen über Aberrationen des Y-chromosoms von Drosophila melanogaster," *Zeit. ind. Abst. Vererb.*, **51**, 253–353 (1929).

OBSERVATIONS ON THE STRUCTURE OF LIVING SALIVARY GLAND CHROMOSOMES IN SCIARA

BY W. L. DOYLE AND C. W. METZ

DEPARTMENT OF EMBRYOLOGY, CARNEGIE INSTITUTION OF WASHINGTON AND ZÖOLOGICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY

Communicated January 15, 1935

Recent investigations on the giant chromosomes of dipteran salivary gland cells by Heitz and Bauer, Painter, Koltzoff and others[1] have focused attention on several problems for the study of which these chromosomes are particularly favorable. One problem has to do with the internal organization of the chromosomes in relation to genes. Koltzoff and Bridges have both interpreted the structures visible in fixed (Koltzoff also in living) material from this point of view.[2]

Owing to the unusual size and nature of the chromosomes under consideration it seems particularly desirable to determine the nature of their organization in the living condition, and to ascertain how accurately the fixation images reflect this organization. The present paper is devoted to this topic. It is based on a study of conditions in the fungus flies (*Sciara*) which are unusually favorable for the purpose.

The living cells, in the intact, uninjured gland, have been examined in body fluid, mineral oil, isotonic Ringer's solution and in various hypo- and hypertonic solutions, and have been observed under the microscope at high magnification during the process of fixation in various ways. The following account summarizes the findings. A more extended treatment will be published elsewhere.

Gross Features.—In normal living glands mounted in body fluid surrounded by mineral oil, the nuclei are spherical and bear a fairly constant size relation to the cells containing them. In mammalian Ringer's solution both cells and nuclei swell, indicating hypotonicity of the solution. In hypertonic salt solutions cells and nuclei immediately shrink. In isotonic salt solution they remain, for a short time, essentially unchanged, and appear as they do in body fluid surrounded by mineral oil.

In body fluid, or isotonic salt solution, as described, the unaltered living chromosomes are invisible, even with the best means of illumination at our disposal. No indication of the transverse segments (bands, discs or rows of granules) is to be seen. The nucleus appears to be optically homogeneous except for small, irregular patches of granular material which apparently represent nucleolar or "chromocenter" material. Careful measurements and estimates place the amount of this material at probably not over ten per cent of the volume of the nucleus. For the most part this material appears to be distributed in thin layers which serve to outline contorted regions or tracts which are optically empty. As will be shown below, the latter represent the chromosomes. They are more or less cylindrical except where distorted by pressure against the nuclear membrane or one another. Relatively little material lies between them. In other words, hardly any nuclear sap or karyolymph is detectable. The optically empty material (chromosomes) appears to comprise eighty to ninety per cent of the nuclear content.

Modification of the Chromosomes.—When normal nuclei, as just described, are injured physically or with certain chemicals, or begin to degenerate, two conspicuous changes occur The chromosomes shrink in size and the transverse bands or segments become visible. The degree of change varies according to the nature of the modifying agent. Aceto-carmine fixative, or forty-five per cent acetic acid, causes a sudden and violent contraction of the chromosomes, with a shrinkage in volume estimated at from fifty to seventy per cent. This may readily be observed under the microscope as it occurs. It is seen best when the chromosomes are made visible by the use of a slightly hypertonic salt solution before the fixative is added. The hypertonic solution causes a slight shrinkage of the chromosomes, but not enough to mask the extreme effects of the acetic acid.

Moderate changes produced by hypertonic salt solutions may be reversed by addition of hypotonic solutions. This reversion may be repeated several times with the same preparation and the changes may be followed continuously under the microscope. Introduction of slightly hypertonic solution results in the gradual appearance of the transverse bands or discs within the chromosomes, accompanied by accumulation of clear material around each chromosome. This reveals the outlines of the chromosomes and shows that they occupied the regions previously optically empty. With increasing shrinkage the transverse bands, which at first appear to be smooth and relatively thin, become more irregular, granular and thickened. Hypotonic solution, if applied soon enough, reverses the process and restores approximately the original appearance.

Chromosomes in glands of young larvae behave in essentially the same manner as those of prepupae, except that in the latter the bands are pro-

duced by smaller changes in the medium and the number of times the banded (dehydrated) appearance may be reversed to give the homogeneous (hydrated) appearance is smaller in older specimens than in younger ones.

When living, normal cells are fixed in ten per cent formalin instead of acetic acid, under observation, much less alteration in size or structural characteristics of the chromosomes is observed. Shrinkage is moderate instead of extreme, and the transverse bands are relatively smooth, straight and thin, as compared with the more granular and irregular ones seen after acetic fixation. Subsequent treatment of these formalin preparations with acetic acid gives results resembling those following acetic alone, with gradations depending on the time of original exposure to formalin (one minute to several hours).

Flemming and Bouin fixatives result in fixation images similar to those produced by acetic acid, although shrinkage of the chromosomes is less extreme in Bouin then in Flemming.

Fixation in osmic acid solution differs from either of the types just described. Osmic acid in isotonic saline solution fixes the chromosomes without appreciable alteration in size and gives them a finely granular appearance. Subsequent treatment with acetic acid does not greatly alter the proportions of segments or matrix.

Mercuric chloride fixation results in a violent shrinkage of nucleus and chromosomes, and in coagulation of the fluid lost from the chromosomes in the process of shrinking. The latter fact indicates that the extruded fluid is not simply water and inorganic salts.

Exposure to ultra-violet radiation, or to the intense visible light ordinarily used with high powers of the microscope, results in changes similar to those produced by acetic acid, as described above, although they take place more slowly.

The observations summarized above indicate that the chromosomes under consideration differ in consistency and make-up from ordinary prophase or metaphase chromosomes in dividing cells, for the latter do not occupy such a large proportion of the nucleus and do not exhibit such extreme shrinkage in volume after acetic fixation. The observations also suggest the possibility that in the normal living state the segments (presumably representing the chromioles) may all be disc-like and that the granules which have been considered to represent genes may appear as a result of fixation or other injury. The evidence supports the suggestion of Metz and Gay[2] that the protoplasm within the segments differs from that between them, and that different segments likewise differ from one another in this respect. It is apparently the intersegmental material which exhibits most of the shrinkage after acetic fixation—thus making the segments biscuit-like or "capsular" in shape.

[1]References have been given in detail by Metz and Gay in these PROCEEDINGS, **20**, 617–621 (1934). See also Bridges, *Amer. Nat.*, **69**, Jan.-Feb. (1935); Painter, *Ibid*, Jan.-Feb. (1935); Metz and Gay, *Science*, **80**, 595–596 (1934).

[2]See Metz and Gay, these PROCEEDINGS, loc. cit., for discussion.

GENETICS OF SENSORY THRESHOLDS: VARIATIONS WITHIN SINGLE INDIVIDUALS IN TASTE SENSITIVITY FOR PTC[1]

BY THEODORA NUSSMAN SALMON AND ALBERT F. BLAKESLEE

HUNTER COLLEGE, N. Y., AND CARNEGIE INSTITUTION OF WASHINGTON, DEPARTMENT OF GENETICS, COLD SPRING HARBOR, N. Y.

Communicated January 12, 1935

Not only is the taste world of one individual different from that of another[2] but it now appears that the taste world of any one individual is not a stable one. Because of its threshold range among individuals, PTC (Phenyl Thio Carbamide) has become a convenient reagent for testing taste reactions. Evidence has already been published[3] which shows that the ability of some and inability of others to taste the crystals of PTC, as well as taste acuteness for solutions of different concentration, are due to innate hereditary differences in the individuals tested. The present paper gives results from a study of the methods used in determining individual taste thresholds for PTC in preparation for investigation of taste thresholds for a number of unrelated substances.

The persons tested were all adults, members of the secretarial and technical staff of the Department of Genetics. The same number or letter has been retained for each individual throughout. Concentrations of PTC were used from 1:5120M up to 1:312.5 using a factor of two, such that each solution used in a test was twice as concentrated as that previously administered. In each test approximately 0.6 cc. was given by means of the straw method.[4]

Figure 1 shows the distribution of thresholds for 94 persons. In this graph each person is represented by a single record taken during the past summer. The curve is at least skewed toward the right and shows a second mode at 1:625. The curve is irregular, as would be expected from such small numbers. How pronounced the bimodality would appear if a larger population were tested is uncertain. A similar bimodality is indicated by earlier records[3] by one of us in which a factor of 4 instead of 2 was used in making up the test solutions and in which those who could not taste the crystals were classified as negative without threshold determinations with concentrated solutions, which may be tasted by those to whom the crystals are tasteless. These earlier records totaled 433 individuals, only a very few of whom are included in figure 1.

To determine whether the correct quantity was being used to insure the perception of a threshold solution by the subject, twelve individuals whose thresholds had been determined previously with 0.6 cc. were tested with increasing amounts of the dilution just below the threshold dilution (i.e., half as strong). Table 1 shows the results. In no case could a half threshold concentration be tasted even when the quantity was increased to a dosage of as much as 10 cc. Some individuals can taste as little as 0.2 cc. of a threshold solution (i.e., the concentration tasted in 0.6 cc.). Five persons kept 5 cc. of a half threshold solution in the mouth exactly one minute without taste, but immediately afterward all reported a taste within a few seconds when given 0.6 cc. of a threshold solution. It would appear, therefore, that 0.6 cc. in a straw is an adequate quantity to use in tests, since the threshold was not lowered by giving larger amounts.

PTC has the widest range of thresholds of any substance so far studied. For this reason perhaps it is more likely to show a "Twilight Zone" of

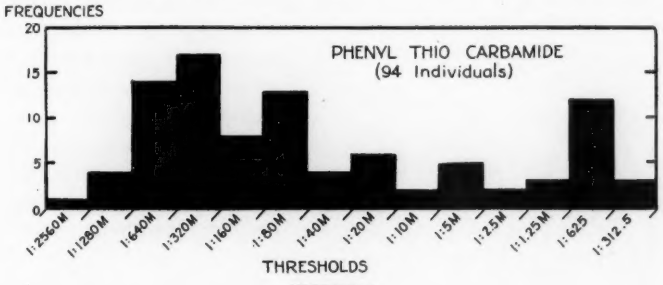


FIGURE 1

several dilutions before a distinct threshold is reached. Taste impressions in this twilight zone are uncertain and include sweet, metallic, salt, sour, astringent and sensations which the subjects recognize as different from water, but which they cannot name or describe.

Changes in temperature of solutions appeared to have no material influence on thresholds. Using solutions of half threshold, threshold and twice threshold at about 8°C., room temperature and about 55°C., no lowering of threshold was observed. Reaction to cold solutions was delayed in some cases. Apparently the solution had to be raised toward mouth temperature before it was tasted.

In a test of the possible influence of the saliva, the tongue was dried with blotting paper and rinsed with water. In no case was a subject able to detect on a blotted or rinsed tongue a solution which under ordinary circumstances was slightly below his threshold. Of 6 subjects who were showing relatively constant thresholds, 2 did not appear to lose their sensitivity to their threshold concentrations, but 4 were rendered less

TABLE 1

SUBJECT NUMBER		HALF THRESHOLD CONCENTRATION								THRESHOLD CONCENTRATION			
		CONC.	0.2 cc.	0.4 cc.	0.6 cc.	0.8 cc.	1 cc.	2 cc.	5 cc.	10 cc.	CONC.	0.2 cc.	0.4 cc.	0.6 cc.
4	♀	1:1250	−	−	−	−	−	−	−	−	1:625	−	+	
41	♀	1:1280*M*	−	−	−	−	−	−	−	−	1:640*M*	−	−	++
12	♀	1:10*M*	−	−	−	−	−	−	−	±	1:5*M*	−	±	+ Met.
23	♀	1:160*M*	−	−	−	−	−	−	−	±	1:80*M*	+	++	
3	♀	1:2500	−	−	−	−	−	−	−	−	1:1250	−	++	
13	♀	1:5*M*	−	−	−	−	−	−	−	−	1:2500	−	+	++
31	♀	1:650*M*	−	−	−	−	−	±	−	−	1:320*M*	±	±	++
5	♀	1:1250	−	−	−	−	−	−	−	−	1:625	±	±	++
10	♀	1:1250	−	−	−	−	−	−	−	−	1:625	±	±	+
45	♀	1:1280*M*	−	−	±	−	−	−	−	−	1:640*M*	±	+	++
W	♀	1:640*M*	−	−	−	−	±	−	−	−	1:320*M*	+	+	++
32	♀	1:640*M*	−	−	−	−	−	−	−	±	1:320*M*	+	+	++

Key: − no taste; ± different from water, cannot describe as a taste; + slight bitter; ++ medium bitter; Met. metallic.

sensitive after the treatment, the extent to which the thresholds were raised varying considerably among the 4 subjects. The time of recovery varied from about a half hour to several hours.

Some individuals perceive the bitter in PTC at once when the threshold concentration is reached, others require several seconds before the taste is noticed. In some the taste is fleeting, in others it persists for the rest of the day.

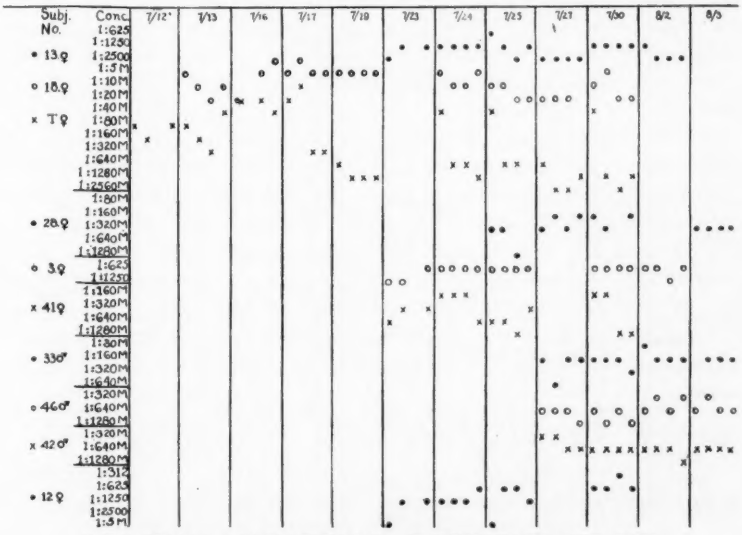
The various subjects have reported all parts of the tongue as responding to bitter stimuli. Some taste it chiefly in the front; others in the middle, sides or back; some in the throat, of whom some include the uvula. At concentrations above threshold, subjects have difficulty in reporting any particular area as most sensitive.

There was at least no strong correlation between the use of tobacco and sensitivity to PTC.

Various experiments indicated that some persons could not be relied on to keep the same threshold from one test to another. This past summer ten people were tested four times a day over a period of from four to nine days: in the morning upon arriving at work, before lunch, after lunch and late afternoon. Figure 2 shows the threshold variations of members of the group within the periods indicated. Subject No. 3, with a factor of 2 between the lowest and highest thresholds,

was the most nearly uniform, although subjects Nos. 13, 46 and 42, who varied by a factor of 4 between extreme records, were relatively constant; subjects Nos. 18, 28, 41 and 33 varied by a factor of 8; subject No. 12 varied by a factor of 16. Subject *T* was the most variable person yet discovered in taste thresholds, with a factor of 256 between extremes. On three occasions she could detect bitter in dilutions of 1:2560*M*, but once was not able to taste the solution until it was 256 times as strong, or 1:10*M*.

Figure 3 shows the taste curves during one day for 7 individuals. Tests were made every fifteen minutes after meals, and at least every hour during the remainder of the day. The subjects were asked not to eat between



INDIVIDUAL VARIATION IN THRESHOLDS FOR PTC

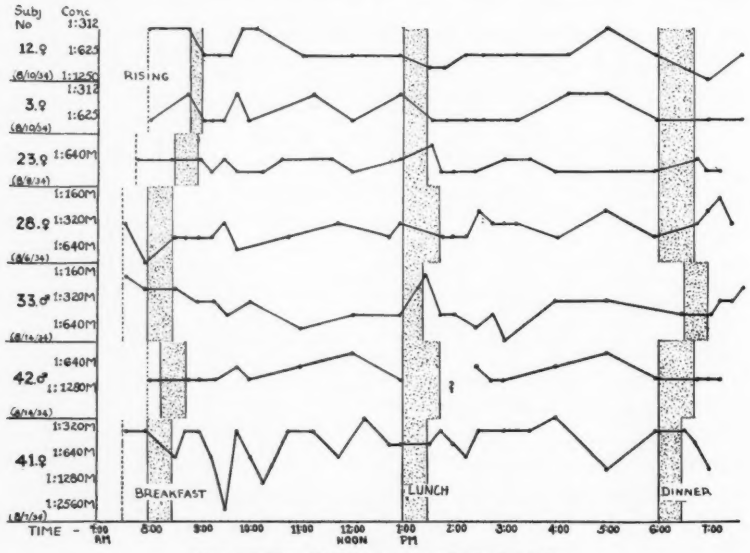
FIGURE 2

meals during the day's test. In addition to the regular dilutions made up on the basis of a factor of two, extra solutions half-way between these were inserted with the purpose of detecting any possible rhythm more easily. Differences were observed after so short an interval as fifteen minutes. Thus, subject No. 41 at 9:30 A. M. could detect PTC in a dilution of 1:2560*M*, but fifteen minutes later needed a 1:320*M* solution, which is eight times as strong. Even when the intervals have been made as short as five minutes, there has been no regularity noted in direction of changes after short intervals, which might suggest taste fatigue or an increased reaction from continued or prolonged stimulation. Fatigue is marked in the case of odors, but in our experience with taste this factor

apparently may be neglected as at most of relatively minor importance.

Henderson and Millet[5] have shown from a study of twelve subjects that the pH of saliva follows a regular daily rhythm, rising during meals and falling again shortly thereafter. The pH of the saliva has earlier been shown by one of us[3] to have no close connection with taste acuity. The lines of taste variation in no way suggest any connection with the rhythmic change in pH of the saliva observed by the authors cited, but the pH of the subjects of figure 3 were not tested.

The number of individuals tested and the limited period of time during



PTC TESTS DURING SINGLE DAYS

FIGURE 3

which the tests were continued were not sufficient to give an adequate sample of the range of variability which people are capable of showing at different times. Schrijver[6] reported that for one person who was tested ten times on successive days, the threshold concentration for quinine hydrochloride varied between 0.005 and 0.015 gram per liter and for saccharine, between 0.010 and 0.0175 gram per liter. These thresholds have the factors between extremes of 3 and $1^3/_4$, respectively, and indicate that the subject was relatively uniform in reactions to the two substances used in comparison with our subjects who were tested with PTC. Such great differences as we have found (from factor of 2 to factor of 256) in single individuals appear not to have been reported before in the literature.

Other tests than those shown in the graphs confirm our belief in the reality of variability of the same subject in respect to threshold reactions taken at different times. One person, for example, who could not taste crystals in the summer of 1933 was found to be able to taste crystals in the summer of 1934 and to detect a definite bitter in a 1:20*M* solution.

Subject No. 23 held a threshold of 1:80*M* consistently for two weeks in July, while numerous tests were made. After an interval of two weeks another series of tests showed a consistent threshold of 1:320*M*. In the same subject, after a second interval of two weeks, the threshold was found to be 1:160*M*. Other subjects, e.g., Nos. 3 and 42, showed very little variation in the threshold from time to time, a quite consistent, slightly varying range being held all summer. As to the fact of variability in thresholds of a single subject there can be no longer any doubt and, as Schrijver[6] has pointed out from fewer data, this variability renders unnecessary the extreme refinement in method recommended by some individuals for determining taste thresholds.

The reason for the variations is not clear. An inspection of the graphs as well as inquiry of the subjects would seem to eliminate the possibility of a regular rhythmic change in taste sensitivity associated with routine daily habits or with regular periodic changes in their physiology. It is our belief, however, that the changes observed in taste thresholds are in some way connected with differences in physiological states. If this should prove to be the case it might be possible to use taste thresholds as an index of unrecognized internal conditions. Such an index might prove to be of clinical value.

[1] Paper presented before Section I (Psychology) at the Pittsburgh Meeting of the A.A.A.S., Dec. 27, 1934.

[2] Blakeslee, A. F., and Fox, A. L., *J. Hered.*, **23,** 97–107 (1932).

[3] Blakeslee, A. F., these PROCEEDINGS, **18,** 120–130 (1932); Snyder, L. H., *Science, N. S.*, **74,** 151–152 (1931); and *Ohio Jour. Sci.*, **32,** 436–440 (1932).

[4] Blakeslee, A. F., and Salmon, M. R., *Eugenical News*, **16,** 105–108 (1931).

[5] Henderson, M., and Millet, J. A. P., *J. Biol. Chem.*, **75,** 559–566 (1927).

[6] Schrijver, F., *Zeitschr. f. Psych.*, **130,** 385–392 (1933).

GENETICS OF SENSORY THRESHOLDS: INDIVIDUAL TASTE REACTIONS FOR DIFFERENT SUBSTANCES[1]

BY ALBERT F. BLAKESLEE AND THEODORA NUSSMAN SALMON

CARNEGIE INSTITUTION OF WASHINGTON, DEPARTMENT OF GENETICS, COLD SPRING HARBOR, AND HUNTER COLLEGE, NEW YORK

Communicated January 14, 1935

In the immediately preceding paper, the present authors have discussed some of the methods used in determining taste thresholds of phenyl thio carbamide (PTC). They have pointed out some of the ways in which people differ in taste reactions one from the other and have reported the discovery that the same person may differ considerably in his taste thresholds from one time to another. These findings apply with certainty only to the PTC used in the tests. Some evidence is available, however, which makes reasonable the natural assumption that they are of rather general application. For references which lead to the literature pertinent to our present discussion, the reader may refer to the preceding paper.

Taste sensitivity to PTC has been found to have an hereditary basis. There were indications from an earlier study of 21 persons at the Department of Genetics that a single individual might have a high taste threshold for one substance and a low threshold for another substance. It seemed desirable, therefore, to make a survey of taste reactions for a number of different substances in order to be able to make a selection of those best adapted for use as reagents in ultimate genetic studies on the taste reactions of humans.

It was realized that the records would be more comparable if all the substances were tested on the same individuals. The tests this past summer were more extensive than those recorded here both as to number of substances used and as to number of people tested with each substance. We are finally able to report full reactions of 47 individuals to 17 different substances. The individual thresholds were obtained each at a single sitting. The previous paper shows that if each threshold had been determined at another time the results assembled in table 1 would have been somewhat different. Our general experience with PTC suggests that most individuals vary in their taste reactions to this substance only within somewhat narrow limits. Until tests have been made on a larger number of people and for extended periods of time, the limits of variation of a single subject will be uncertain. The graphs of figure 1 may give some idea of the maximum limits within which a single person may vary unless there are seasonal changes affecting the form of threshold distribution.

Figure 1 shows graphs of threshold distributions of the same 47 individuals for the 17 substances. The thresholds shown at the base line run from a dilution of 1:5120M of Optochin Base tasted by one subject

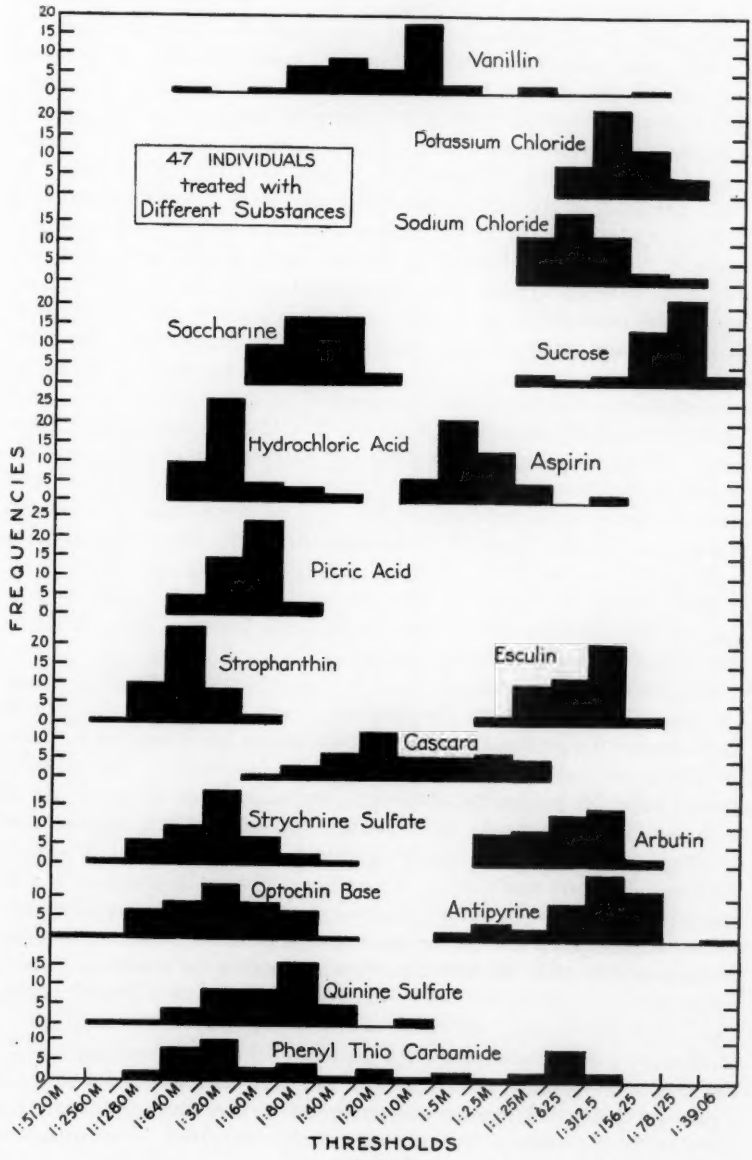

47 INDIVIDUALS
treated with
Different Substances
Vanillin
Potassium Chloride
Sodium Chloride
Saccharine
Sucrose
Hydrochloric Acid
Aspirin
Picric Acid
Strophanthin
Esculin
Cascara
Strychnine Sulfate
Arbutin
Optochin Base
Antipyrine
Quinine Sulfate
Phenyl Thio Carbamide
FREQUENCIES
1:5120M
1:2560M
1:1280M
1:640M
1:320M
1:160M
1:80M
1:40M
1:20M
1:10M
1:5M
1:2.5M
1:1.25M
1:625
1:312.5
1:156.25
1:78.125
1:39.06
THRESHOLDS

FIGURE 1

to a concentration of 1:39.06, which is the high threshold for sucrose and antipyrine. In all cases a factor of 2 was used in making dilutions. In certain cases, particularly when a subject could not taste a saturated solution, he was credited in the diagrams and tabulations as having the next higher threshold, as explained in legend of table 1. In table 1 grades are used for convenience of tabulation in place of the threshold concentrations to which they corresponded. Thus, for PTC grade 1 would include the least acute tasters and correspond to a threshold solution of 1:312.5 while grade 13 would include the most acute tasters and correspond to a concentration of $1:1280M$. These grades correspond only to the 47 subjects tested. If a larger population were studied and the curve were extended a single grade in both directions, grade 1 would be at 1:156.25 and grade 15 would be at $1:2560M$. The spread of the curve may be expressed by the number of grades or by the factor between the two extremes. The latter may be computed by the formula 2^{n-1}, where n equals the number of grades. Thus the PTC distribution in figure 1 embraces 13 grades with a factor difference between the extremes of 4096, which means that the least acute taster must have the solution 4096 times as strong as the most acute taster in order to perceive bitter in this substance. In the larger group represented in the previous paper, the factor difference is 8192.

The substances of figure 1 may be briefly discussed, beginning with the bitters. In this study, indiscriminations between different tastes were noted but not investigated further.

PTC had the greatest range of threshold so far discovered with 13 grades for the 47 subjects and the thresholds are very definite. It is discussed above and in the previous paper. Quinine sulfate had a range of 9 grades. An astringent feeling sometimes preceded the threshold. Two subjects tasted sweet in subliminal dilutions. Optochin Base is a relative of quinine. It had a range of 8 grades with an extreme threshold of $1:5120M$. An astringent feeling accompanied the threshold in several subjects. Cascara had a range of 8 grades. Five could not taste a saturated solution and were listed as in grade 1 with the next higher threshold. To the higher grade tasters, cascara had a clean bitter taste, but to those of lower grade, other tastes frequently accompanied the threshold. Antipyrine had a range of 8 grades. Several felt a subliminal astringency. One could not taste a saturated solution and was listed in grade 1. Strychnine sulfate had a range of 7 grades. Subliminal tastes were given as sweet, salty, sourish, astringent and "skunk." Arbutin had a range of 5 grades. Subliminal impressions were often sweet or sour. Strophanthin had a range of 5 grades. Esculin had a range of 5 grades. Two classed in grade 1 were negative to saturated solution. Picric acid had a range of only 4 grades, but the threshold is generally very definite.

TABLE 1

FORTY-SEVEN INDIVIDUALS AND THEIR TASTE GRADES FOR SEVENTEEN SUBSTANCES

Subject Numbers	PTC	Quin	OpBs	Casc	AnPy	Stry	Arbt	Strph	Escl	Picr	Aspr	HCl	Sucr	Sach	NaCl	KCl	Vanl
Most Acute	1:1280M	2560M	5120M	160M	5M	2560M	2.5M	2560M	2.5M	640M	10M	640M	1.25M	160M	1.25M	625	640M
1♂	1	4	4	3	4	2	2	2	2	3	1	1	2	2	3	1	4
●2♂	1	4	4	5	4	4	*5	5	3	3	5	3	3	3	4	*4	9
3♀	2	3	2	5	4	4	3	3	2	3	4	4	3	3	3	3	7
●4♀	2	3	4	5	5	5	2	3	3	2	4	4	1	2	*5	*4	7
5♀	2	3	5	5	5	4	3	3	2	2	5	4	*6	*4	2	3	9
6♀	2	4	3	3	4	4	4	2	2	2	5	4	3	2	3	3	7
7♀	2	4	3	4	3	2	2	1	1	2	5	3	1	2	3	1	8
●8♂	2	4	4	4	3	4	*5	4	4	2	3	4	3	1	2	2	9
9♂	2	5	2	5	3	3	2	3	3	2	4	4	*6	2	4	3	9
10♀	2	6	5	5	6	4	3	3	3	2	4	*5	2	3	*5	3	7
11♀	3	4	3	*8	5	5	2	3	3	2	*6	*5	2	3	4	2	7
●12♀	3	4	4	1	5	5	3	2	2	2	4	4	4	2	*5	3	7
13♀	4	6	5	2	7	5	4	3	3	3	5	*5	2	3	4	2	9
14♂	5	5	3	4	4	4	3	3	4	2	4	4	2	3	3	2	7
15♂	5	6	2	2	3	4	2	3	2	2	5	*5	2	3	3	*4	10
●16♀	6	8	4	6	4	6	4	3	3	*4	5	4	5	*4	4	1	7
17♂	7	1	2	4	3	1	1	3	2	2	1	1	1	2	1	1	7
18♀	7	3	4	2	4	4	2	4	2	3	5	4	3	2	4	3	7
●19♂	7	4	3	5	3	6	2	3	1	3	4	4	2	*4	4	3	1
●20♀	8	4	1	3	3	4	3	1	2	*4	4	4	2	3	4	3	7
●21♂	9	1	2	2	3	4	4	3	2	*4	3	2	2	2	3	2	10
22♀	9	5	3	6	4	5	2	3	2	3	5	*5	4	*4	4	2	10
23♀	9	5	6	6	5	4	4	3	2	3	5	4	2	*4	*5	*4	4
24♀	9	6	6	3	3	3	3	4	2	2	5	4	2	3	4	3	7
●25♂	10	4	5	1	4	4	3	3	2	2	5	4	3	3	*5	3	6
26♂	10	4	5	5	4	6	2	4	3	2	5	*5	5	2	*5	2	7
●27♂	10	4	6	1	4	5	3	3	3	2	*6	3	2	*4	3	2	7
28♀	11	4	4	6	3	4	3	3	2	3	3	*5	2	3	4	3	*13
29♂	11	4	5	7	5	2	*5	3	*5	2	4	2	3	2	4	3	7
30♀	11	5	4	4	4	4	2	2	2	1	4	4	2	2	3	2	6
31♀	11	5	4	6	4	4	*5	3	3	3	5	*5	2	2	4	3	7
32♀	11	5	6	3	7	6	3	4	3	3	*6	4	3	*4	*5	3	9
33♂	11	6	2	5	4	4	3	4	4	2	4	2	2	1	3	1	8
●34♀	11	6	3	3	4	3	*5	2	*5	1	5	4	2	2	3	3	9
●35♀	11	6	4	4	4	3	1	2	2	2	*6	4	3	3	4	*4	10
●36♂	11	6	6	1	6	4	4	2	4	3	4	*5	3	3	*5	2	11
37♀	11	7	5	5	5	3	4	3	4	3	5	*5	*6	2	*5	3	8
38♂	12	3	2	6	3	5	2	3	2	2	*6	3	3	*4	3	3	10
39♂	12	4	3	2	5	5	*5	3	3	*4	*6	4	3	3	4	*4	7
40♂	12	4	5	1	1	4	2	2	2	2	5	2	2	2	1	2	8
41♀	12	5	3	6	7	3	3	2	2	2	3	3	2	3	2	3	10
42♂	12	5	4	2	3	3	2	3	2	2	5	4	3	2	4	3	8
43♀	12	6	5	5	*8	5	*5	4	3	2	5	4	4	*4	*5	2	7
44♀	12	7	*8	7	6	*7	4	*5	4	3	3	4	2	3	4	*4	10
45♀	12	*9	7	7	8	6	*5	4	4	*4	5	4	2	3	*5	3	9
●46♂	*13	7	6	2	5	5	4	3	4	3	5	4	3	1	4	*4	9
●47♀	*13	7	6	5	7	6	2	4	4	1	4	4	2	*4	*5	3	8

Subjects arranged according to increasing acuteness in taste for PTC and succeeding substances. Grade 1 represents the least acute tasters for a given substance. Grade 2 represents those with a threshold at half the concentration of the previous grade and so on to the highest grades which represent the most acute tasters. The highest grades for a given substance are starred in the vertical column below each substance and the concentrations represented by such grades are shown in the second horizontal row. Stars in circles are placed against the 15 subjects who tested in the lowest grade for one substance and in the highest grade for another substance. In the case of cascara, antipyrine and esculin, a few subjects were negative to a saturated solution and were listed as having the next higher threshold and placed in Grade 1. One subject was negative to a 1:80*M* solution of Optochin Base, beyond which concentration it had been decided it would be undesirable to go in tests. She was credited with a threshold of 1:40*M* and placed in Grade 1.

Of the sours, aspirin had a range of 6 grades. It tastes sour to most, though a few find a salty and more a bitter accompaniment. HCl had a range of 5 grades. Sweet, salt, bitter and astringent were accompanying sensations reported.

The sweets are difficult to grade since the taste is often fleeting. Sucrose had a range of 6. Among the accompanying impressions reported are: fragrant, fruity, sour, astringent, slippery, with salty and sour after-tastes. Saccharine had a range of 4 grades. Astringency also reported.

Among the salty substances NaCl had a range of 4 dilutions. Sweet, sour, bitter and soapy are reported subliminal impressions. KCl had a range of 4 dilutions and is extremely unpleasant to most people, who described it as sickening. A wide range of accompanying sensations were reported.

Vanillin had a range of 13 grades. It was variously described as sweet or as like some substance such as chocolate, in which it is a flavor. It is uncertain whether the impressions recorded are to be classified as taste or smell. It was included to see if reactions to a flavor could be tested by the straw method.

In our earlier work as well as in that of others, those who could not taste the commercial crystals were called "non-tasters" and were classified as "taste blind" or taste deficient. This we believe unfortunate. An inspection of the graphs will show that for all substances there is a continuous series of thresholds between the highest and lowest grades of tasters. With solutions it is arbitrary at what threshold concentration the population is divided into "tasters" and "non-tasters." With PTC, which is only slightly soluble in water, this separation can be made on the basis of a small amount of the dry material which comes commercially in certain sized crystals. By using finely divided crystals obtained by passing paper through alcoholic solutions of different concentrations we are able to grade thresholds and change into "tasters" individuals who would be classed as "non-tasters" of the commercial product. A similar condition is found with other substances of poor solubility in water. Thus, exactly half of 28 subjects were negative to a small quantity of powdered theobromine, but when a larger quantity was used more were tasters. With saturated solutions, 11 were negative out of 53 tested.

Figure 1 has given the frequency distributions of thresholds for the 17 substances tested. Table 1 gives all the threshold grades for each of the 47 subjects. The substances given by abbreviations in the top row are in the order of their discussion in preceding paragraphs. It will be seen that although there is a tendency for those who are acute tasters for PTC to be also acute tasters for other substances and vice versa, there are many individual exceptions. Fifteen out of the 47 subjects were in the lowest grade for one substance and in the highest grade for another substance. These are indicated by a star in a circle preceding their number.

TABLE II

TASTE GRADES FOR PTC AND CORRESPONDING MEAN GRADES FOR OTHER SUBSTANCES

PTC GRADE	NO. IN GRADE	OTHER SUBSTANCES																
		QUN	OPB	CAS	APY	STR	ARB	STP	ESC	PIC	ASP	HCL	SUC	SAC	NACL	KCL	VAN	AVER.
1	2	4.0	4.0	4.0	4.0	3.0	3.5	3.0	2.5	3.0	3.0	2.0	2.5	2.5	3.5	2.5	6.5	3.3
2	8	4.0	3.5	4.5	4.1	3.8	3.0	2.8	2.4	2.1	4.3	4.0	3.1	2.4	3.4	2.8	7.9	3.6
3	2	4.0	3.5	4.5	5.0	5.0	2.5	2.2	2.5	2.0	5.0	4.5	3.0	2.5	4.5	3.5	7.0	3.8
4	1	6.0	5.0	2.0	7.0	5.0	4.0	3.0	3.0	3.0	5.0	5.0	2.0	3.0	4.0	2.0	9.0	4.3
5	2	5.5	2.5	3.0	3.5	4.0	2.5	3.0	3.0	2.0	4.5	4.5	2.0	3.0	3.0	3.0	8.5	3.6
6	1	8.0	4.0	6.0	4.0	6.0	4.0	3.0	3.0	4.0	5.0	4.0	5.0	4.0	4.0	1.0	7.0	4.5
7	3	2.7	3.0	3.7	3.3	3.7	1.7	3.3	1.6	2.7	3.3	3.0	2.0	2.7	3.0	2.3	5.0	2.9
8	1	4.0	1.0	3.0	3.0	4.0	3.0	1.0	2.0	4.0	4.0	4.0	2.0	3.0	4.0	3.0	7.0	3.3
9	4	4.3	4.3	4.2	3.8	4.0	3.2	3.3	2.4	3.0	4.5	3.8	2.3	3.3	4.0	2.8	7.8	3.8
10	3	4.0	5.3	2.3	4.0	5.0	2.7	3.0	2.6	2.0	5.3	4.0	3.3	3.0	4.3	2.3	6.7	3.7
11	10	5.4	4.3	4.4	4.6	3.7	3.5	2.8	3.4	2.3	4.6	4.0	2.8	2.4	4.0	2.7	8.8	4.0
12	8	5.3	4.6	4.5	5.1	4.8	3.5	3.3	2.7	2.6	4.8	3.5	2.6	3.0	3.5	3.0	8.6	4.1
13	2	7.0	6.0	3.5	6.0	5.5	3.0	3.5	4.0	2.0	4.5	4.0	2.5	2.5	4.5	3.5	8.5	4.4
1–9	24	4.3	3.5	4.1	4.0	4.0	2.9	2.9	2.4	2.6	4.2	3.8	2.7	2.8	3.6	2.6	7.3	3.6
10–13	23	5.4	4.7	*4.1*	4.8	4.4	3.4	3.1	3.1	*2.3*	4.7	*3.8*	2.8	*2.7*	3.9	2.8	8.4	4.0
1–2	10	4.0	3.8	4.2	4.0	3.3	3.2	2.9	2.4	2.5	3.6	3.0	2.8	2.4	3.4	2.6	7.2	3.5
12–13	10	6.2	5.3	*4.0*	5.5	5.1	*3.2*	3.4	3.3	*2.3*	4.6	3.8	*2.5*	2.7	4.0	3.2	8.5	4.3

The relationship between taste for PTC and that for other substances may be shown by table 2. If the population is separated into two halves on the basis of grades for PTC the group with the higher PTC grades will in most cases have a higher mean grade for other substances and vice versa. There are four exceptions, however, which are represented by cascara, picric acid, HCl and saccharine. A more equitable comparison is obtained by using the grades 1–2 against grades 12–13 since these groups contain the same number of subjects. Here again there are 4 exceptions, which in two cases are not the same as in the other comparison. It may be concluded that the correlation between acuteness of taste for two substances, though generally positive is relatively low, with many striking exceptions.

Intensity of sensations cannot be accurately measured by thresholds though they may be used as an index of taste acuteness. With this reservation in mind, some interesting comparisons are possible from table 1. To one subject, PTC was 16 times as bitter as quinine, as determined by relative thresholds; to another, quinine was 256 times as bitter as PTC. To one, cascara was twice as bitter as quinine; to another, quinine was 256 times as bitter as cascara. Saccharine, to one, was 32 times as sweet as cane sugar; to another, 2000 times. No two of the subjects were alike in all their thresholds. They form a group of "47 Varieties of People." If enough substances were used as test reagents, it might be possible to show that no two people are alike in their sensory reactions. If one could express these difference as is done in a chemical formula, it is fair to say that no two individuals would have the same sense formula. Each of us lives in a different world and, as shown in the previous paper, each within limits lives in a world which is subject to change without notice.

[1] Paper presented before Section L (Psychology) at the Pittsburgh Meeting of the A.A.A.S., Dec. 27, 1934.

SOMATIC SEGREGATION DUE TO HEMIZYGOUS AND MISSING GENES AND ITS BEARING ON THE PROBLEM OF ATYPICAL GROWTH

BY DONALD F. JONES

CONNECTICUT AGRICULTURAL EXPERIMENT STATION, NEW HAVEN, CONNECTICUT

Communicated December 18, 1934

Recent cytogenetic investigations have shown many ways whereby recessive genes may be brought to expression by the removal of their dominant alleles. Non-disjunction, deficiency, unequal translocation, haploidism and other abnormal chromosome behavior may bring about un-

usual segregation at an infrequent and varying rate. Unpaired genes may be called *hemizygous*. This term has been used to designate the condition in a heterozygous sex in which one chromosome has no homolog and seems applicable to autosomic genes that have lost the other member of the pair whether they were originally heterozygous or homozygous.

An important effect of hemizygous genes lies in the field of somatic segregation. Variations of this kind are easily demonstrated in endosperm and aleurone color characters in maize. Emerson[1] has shown conclusively that mosaic seeds in maize are due to chromosomal aberrations of some kind without deciding definitely what type of irregularity is involved. He believed that non-disjunction was the most likely agency for the reason that linked genes were included in the same mosaic areas.

In a *c sh* stock pollinated by *C Sh*, colorless (*c*) mosaic areas have been obtained by the writer that were not *sh*. These colorless areas are large enough to show clearly the shrunken tissue if it were present. This proves that non-disjunction is not the reason for the appearance of the recessive characters in this material. Additional evidence presented in table 1 indicates that mosaics are more commonly due to deletions. The frequency of various mosaics is shown with the genes involved. The recessive gene in every case came through the ovule while the dominant gene came through the sperm. Where the pollination number is the same the two classes of mosaic seeds came from the same ears.

The frequency of somatic segregation in this material varies from 1 in 29 to 1 in 3676. This is due to the fact that loci in different chromosomes are involved and also to the dissimilarity in the plants cross-fertilized.

The mosaics classified in table 1 involve the *C*, *I*, *R*, *Su* and *Y* genes. All except the first two are located on different chromosomes. Genes *C* and *I* are either allelic or are so close together that no crossovers have been detected. For the purpose of this discussion they can be considered to be at the same locus. The *Cc* mosaics show, in red or purple seeds, in the form of colorless aleurone areas of varying size and shape. The *Ii* mosaics appear as red or purple areas on otherwise colorless seeds, disregarding the endosperm color underneath, which may be yellow or white. On white seeds these colored spots are conspicuous and can be seen when quite small. The individually colored cells can be counted under the microscope. One tiny dot-like mosaic barely visible to the unaided eye covers an area of 61 cells. Single colored cells are numerous in some combinations. Only the macroscopically visible mosaics are counted in the accompanying tabulation.

The frequency of mosaics involving this locus vary from 1:29 to 1:1007. Mosaics involving the *Su* gene vary from 1:222 to 1:1365. Considering only the mosaics from the same plants where the parentage and the environment are the same, in pollination number 4, table 1, the ratio is

1:29 for *I* and 1:395 for *Su*. The *Su su* mosaics, bringing about a change from smooth opaque to wrinkled translucent endosperm cannot be detected as easily as the color changes. Small mosaics probably have no visible effect. If we compare with these only the *I* mosaics that have colored areas as large or larger than the smallest detectable *Su* mosaics we find a ratio of 1:178 for *I* and 1:385 for *Su*. Since these occur under equal conditions the difference is probably significant.

The *Su* gene is on chromosome 4 close to the spindle fibre attachment point. The *I* gene is on chromosome 9 considerably farther removed

TABLE 1

THE FREQUENCY OF MOSAICS DUE TO CHROMOSOMAL ABERRATION IN THE ENDOSPERM OF MAIZE SEEDS

POLLINATION NUMBER	CHROMOSOME NUMBER	GENES INVOLVED	RATIO		NUMBER	
			MOSAIC	NORMAL	MOSAIC	NORMAL
1	9	*Cc*	1	299	4	1196
2	9	*Cc*	1	1007	5	5035
3	9	*Cc*	1	493	2	986
			1	656	11	7217
4	9	*Ii*	1	29	118	3448
5	9	*Ii*	1	56	39	2195
			1	36	157	5643
6	10	*Rr*	1	120	1	120
4	4	*Su su*	1	395	9	3557
7	4	*Su su*	1	222	8	1773
8	4	*Su su*	1	1365	4	5460
			1	515	21	10790
7	6	*Yy*	0	1781	0	1781
9	6	*Yy*	1	1895	1	1895
			1	3676	1	3676

from the spindle fibre. Stadler (unpublished) has suggested that this is a possible reason for the difference in frequency. The farther genes are from the spindle fibre attachment point the more frequently are they lost. If this is the explanation, and holds true for other genes, this is additional evidence that the mosaics are due to deletions rather than non-disjunction.

Only 1 *Yy* mosaic has been found among 3676 seeds examined where the color was clear and the conditions such that mosaics could surely be seen if they occurred. The *Y* gene is on chromosome 6 to which is attached the nucleolus.

The wide differences in the frequency of mosaics in the several pollinations are indications of germinal dissimilarity. Pollination number 4 brings together two distinct types of maize, a large-seeded yellow sweet corn and a small white-seeded pop corn, differing in many characters in the plant and ear. In this case the foreign chromosomes functioning in the unfamiliar cytoplasm are frequently aberrant. Since maize endosperm is triploid tissue, with two sets of chromosomes derived from the ovule and one set derived from the sperm, mosaics involving the disappearance of dominant genes contributed by the female are rarely seen. In 4283 seeds resulting from a CC♀ by c♂ pollination, 9 mosaics with small uncolored areas were found. These show clearly that C has been lost from two chromosomes. The two chromosomes, or parts of chromosomes containing C, are not lost necessarily at the same time. One can be lost from a larger area than that from which both are lost. These mosaics show irregular growth in the tissue including and surrounding the mosaic area.

One red aleurone seed produced on a self-pollinated, homozygous plant had one small definite white spot similar to other red to white mosaics. The only apparent explanation for this uncolored spot is a loss of either A, C or R from all three chromosomes or a mutation from i to I.

In pollination number 5 a red-seeded variety was crossed by a colorless variety carrying an inhibitor for aleurone color, and also the *Pr* gene changing red to purple. This latter factor could not show in the normally uncolored seeds resulting from this cross-pollination, but the mosaic areas were purple in nearly every case due to the action of this color gene. In one mosaic comprising about 30 cells the color was distinctly red. This could only be due to the loss of the *Pr* gene in chromosome 5 accompanying the loss of I in chromosome 9.

A plant homozygous for *Su* and De_{16} was naturally pollinated in an isolated field with pollen from *su* plants segregating de_{16}. In 280 seeds on one ear there were 16 mosaic seeds that showed clear indication of chromosome aberration, a ratio of 1:18. The mosaic areas varied from small dots of aborted tissue to large folded and wrinkled areas involving half of the seeds and were clearly irregular, some mosaic seeds exceeded the size of the normal seeds. These areas were opaque and showed no evidence of being *su* in composition. They may have been de_{16} although they did not show the discoloration characteristic of de_{16}. For this recessive gene to show, the dominant allele from both homologous female chromosomes would have to be lost, an occurrence that happens so rarely as to be very improbable in this case. If this is the explanation it is a case of irregular growth due to the uncovery of a hemizygous, recessive lethal gene.

Another explanation is more probable. Other evidence indicates that there is a small deletion in chromosome 4 in the plants used for the female

parent. This deletion has not been seen cytologically although looked for, and is evidently quite small. The plants produce variable-sized pollen, and aberrant sugary, tunicate ratios and give other clear indications of a deficiency near the *Su* locus. In this case the chromosome suspected of having a deletion came through the ovule. Non-disjunction or a corresponding deletion in the homologous chromosome would eliminate one or more genes from all chromosomes. If this is so we have a clear case of abnormal growth due to missing genes.

Demerec[2] has shown in Drosophila that a deletion in one chromosome followed by a loss of the normal mate in the homologous chromosome has

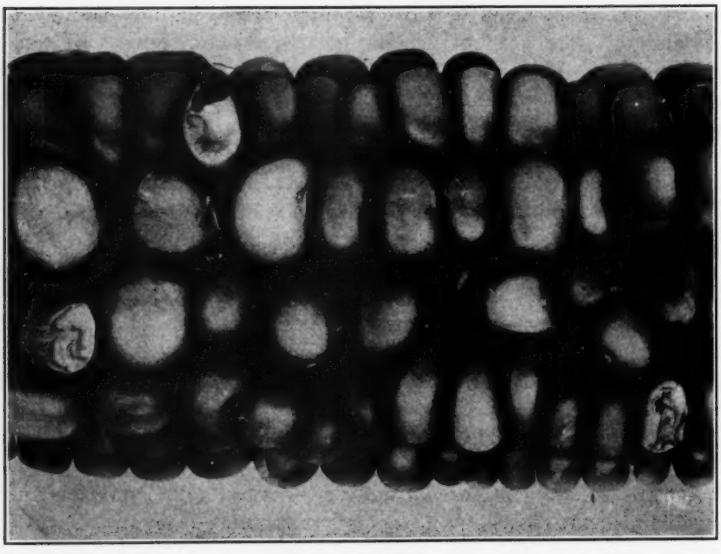


FIGURE 1

Abnormal growth accompanying chromosome aberration.

a lethal action in very small areas of tissue in most cases. Ephrussi[3] using the same material found that similar somatic segregations may be viable.

Kostoff and Kendall[4] have demonstrated irregular chromosome behavior in galls produced by insects. The profound effect that insect, fungus and bacterial parasites can have in the irregular development of plant tissue is well known. Certain varieties of apples are characterized by well-marked mole-like projections on the surface of the ripe fruit, due to the egg-laying activities of the plum curculio (*Conotrachellus nenuphar* Herbst.). The wound is made while the fruits are small. The abnormal

tissue develops with the growing fruit and at maturity presents a distinctly unregulated mass of cells. That this tissue is definitely altered is shown by the fact that the scab fungus (*Venturia inaequalis* (Cke.) Wint.) will spread up to the margin of these over-growths but no farther. Some varieties of apples respond to the stimulus from the insect and others do not and this tendency is transmitted to some of their seedlings.

The writer[5] has called attention to the similarity between the sporadically occurring gall formations and fasciations in plants and atypical growths in animals. The evidence from many sources points clearly to specific chromosome loss as the initial causal agent. Whether they are due to the uncovery of recessive lethal genes in somatic tissue, or to hemizygous normal genes, to missing genes due to the loss of corresponding sections of homologous chromosomes is not as yet known.

The action of x-rays, radium, ultra-violet light, heat and other physical agents in producing chromosomal irregularities is well known. Mottram,[6] in addition to some of the above means, obtained non-disjunction of chromosomes as an effect of treatment with tar and gentian violet. The same result has been obtained with dibenzanthracene and other coal tar derivatives. Barrows, Hieger and Kennaway[7] produced tumors in connective tissue by the use of this same substance. The well-known effect of x-rays, radium, hormones and other powerful physical and chemical agents in bringing about unregulated growths seems conclusively to be due in some way to their action upon the chromosomes resulting in the loss of parts or of whole chromosomes. Both chromosomal aberrations and unregulated growths occur naturally when no known external influence is present. When the loss of chromatin removes genes necessary to regulate cell coördination abnormal growth results. The nature of this atypical growth depends upon the genes removed.

Inherited differences in the frequency of atypical growths may be due to the fact that viable deficiencies or lethal factors persist in certain families, as is known, but have no effect in somatic tissue until the protecting genes in the homologous chromosomes are lost. It is shown here that families differ in the frequency of chromosomal aberrations.

[1] Emerson, R. A., "Genetic Evidence of Aberrant Chromosome Behavior in Maize Endosperm," *Amer. J. Botany*, **8**, 411–424 (1921).

[2] Demerec, M., "Biological Action of Small Deficiencies of X-Chromosome of Drosophila melanogaster," *Proc. Nat. Acad. Sci.*, **20**, 354–359 (1934).

[3] Ephrussi, Boris, "The Absence of Autonomy in the Development of the Effects of Certain Deficiencies in Drosophila melanogaster," *Proc. Nat. Acad. Sci.*, **20**, 420–422 (1934).

[4] Kostoff, Dontcho, and Kendall, James, "Irregular Meiosis in *Lycium halimifolium* Mill. Produced by Gall Mites (Eriophyes)," *J. Genetics*, **21**, 113–115 (1929).

[5] Jones, Donald, F., "The Similarity Between Fasciations in Plants and Tumors in Animals and Their Possible Genetic Basis," *Science*, **81**, 75-76 (1935).

[6] Mottram, I. C., "Some Effects of Cancer-Producing Agents on Chromosomes," *British J. Exper. Path.*, **15**, 71–73 (1934).

[7] Barrows, H., Hieger, I., and Kennaway, E. L., "The Experimental Production of Tumors of Connective Tissue," *Amer. J. Cancer*, **16**, 57–67 (1932).

GENERALIZED MINIMAX PRINCIPLE IN THE CALCULUS OF VARIATIONS

BY G. D. BIRKHOFF AND M. R. HESTENES

DEPARTMENT OF MATHEMATICS, HARVARD UNIVERSITY

Communicated January 12, 1935

1. *Introduction.*—A fundamental problem in the Calculus of Variations is that of establishing the existence of extremals satisfying given initial conditions, and of classifying them. The first general existence theorem involving simple integrals was that of Hilbert. He was concerned with the existence of absolute minima, as have been Tonelli and others following him. In the enunciation of his minimax principle Birkhoff[1] established the existence of extremals of higher type. The subsequent fundamental work of Morse[2] has developed systematically by the use of methods of topology the existence and classification of still more complicated types of extremals. It is part of the aim of the present paper to show that all these further extremals can be obtained by the aid of a natural and simple extension of the minimax principle.[3]

Our abstract formulation of this generalized minimax principle hinges on a suitable definition of extremals of "type k" for a functional $J(P)$ defined on a function space Ω. The definition is a natural one and is constructive in character. At least in many non-singular cases it can be shown to be equivalent to the usual definition. We give a method of counting such extremals of various types. These counts satisfy the inequalities of Birkhoff and Morse. We have essentially reduced the question of existence of extremals of the various types to that of finding a class of deformations D_k, the existence of which in many important cases is intuitively obvious.

2. *Classification of Cycles and Chains.*—Consider a space Ω in which the ordinary concepts of topology, such as k-cycles, k-chains, non-bounding k-cycles, addition (modulo 2), etc., are well defined. The number R_k of independent non-bounding k-cycles in a maximal set of such cycles is called the k-th connectivity of Ω. We admit the possibility of R_k being infinite.

Let $\mathfrak{N}_k$ be a class of k-cycles which is closed under addition and which contains a set of R_k non-bounding k-cycles which are independent on Ω. Let $\mathfrak{M}_0 \equiv \mathfrak{N}_0$ and $\mathfrak{M}_k$ ($k > 0$) be a class of k-chains which is closed under

addition and has the following properties. The class $\mathfrak{M}_k$ contains $\mathfrak{N}_k$ as a sub-class and every k-cycle in $\mathfrak{M}_k$ is in $\mathfrak{N}_k$. The bounding $(k-1)$-cycle of a chain in $\mathfrak{M}_k$ belongs to $\mathfrak{N}_{k-1}$ and every bounding $(k-1)$-cycle in $\mathfrak{N}_{k-1}$ bounds a k-chain in $\mathfrak{M}_k$.

If we denote by M_k, N_k the number of independent k-chains in a maximal set of such chains on $\mathfrak{M}_k$, $\mathfrak{N}_k$, respectively, then the following (not independent) relations are immediate if the numbers M_k $(k = 0, 1, \ldots, m)$ are finite:

$$M_k \geqq N_k \geqq R_k \geqq 0 \qquad (k = 0, 1, \ldots, m) \tag{1}$$

$$M_0 = N_0,\ M_j - N_j = N_{j-1} - R_{j-1} \qquad (j = 1, \ldots, m) \tag{2}$$

$$M_k - M_{k-1} + \ldots + (-1)^k M_0 = N_k - R_{k-1} + R_{k-2} + \ldots + (-1)^k R_0 \tag{3}$$

$$M_k - M_{k-1} + \ldots + (-1)^k M_0 \geqq R_k - R_{k-1} + R_{k-2} + \ldots + (-1)^k R_0, \tag{4}$$

the equality in the last expression holding if and only if $N_k = R_k$. If $M_{k+1} = 0$ then $N_k = R_k$ by (1) and (2). It is clear that if R_k is infinite so also are the numbers M_k and N_k.

3. *Generalized Minimax Principle.*—Now consider a functional $J(P)$ defined on the function space Ω having the properties described in §2. We assume that on each k-chain $J(P)$ has a finite absolute maximum which it attains at a set of points P, called the "*critical* k-set of J on the chain."

For each k-chain C_k we define a set of admissible deformations S_{c_k} of C_k into a k-chain, *which never increase* J and which deform every j-chain $(j \leqq k)$ on C_k admissibly, non-bounding cycles being deformed into non-bounding cyles.

Let D_k denote some class of such admissible deformations S_{c_k} of k-chains into k-chains which deforms independent non-bounding k-cycles into independent non-bounding k-cycles. The class of all k-cycles whose critical k-sets are invariant under D_k will be denoted by[4] $\mathfrak{N}_k$. The set $\mathfrak{N}_k$ is closed under addition if we adjoin the null k-cycle. Let $\mathfrak{M}_0 = \mathfrak{N}_0$ and $\mathfrak{M}_k$ $(k > 0)$ be the class of all k-chains whose boundaries are in $\mathfrak{N}_{k-1}$ and whose critical k-sets are invariant under D_k. Clearly $\mathfrak{M}_k$ and $\mathfrak{N}_k$ are related as in §2, except for the fact that there may exist a $(k-1)$-cycle in $\mathfrak{N}_{k-1}$ which bounds on Ω but bounds no chain in $\mathfrak{M}_k$.

We make the following assumptions concerning the selected class of deformations D_k.

I. *Every k-cycle can be deformed by a deformation in D_k into a k-cycle in $\mathfrak{N}_k$, possibly the null k-cycle.*

II. *Every k-chain on Ω whose boundary is in $\mathfrak{N}_{k-1}$ can be deformed into a k-chain in $\mathfrak{M}_k$ having the same boundary, by a deformation in D_k.*

III. *Every class of admissible deformations containing D_k as a sub-class*

and preserving independence of non-bounding cycles defines the same class $\mathfrak{M}_k$.

Postulate II implies that the set $\mathfrak{M}_k$ is closed under addition. We define the numbers M_k, N_k, R_k as in §2. It is clear that these numbers satisfy the relations (1), (2), (3), (4).

A point P of Ω which belongs to the critical k-set of a k-chain in $\mathfrak{M}_k$ will be called an *extremal of* J *of type* k. The count of all extremals of type k is defined to be M_k. The count of all extremals belonging to the critical k-sets of the k-cycles in $\mathfrak{N}_k$ will be defined to be N_k. Extremals of type k and their counts are well defined by virtue of postulate III. *It should be noted that extremals of type* $j \leqq k$ *are invariant under* D_k *while those of type* $j > k$ *are not unless they are also of type* k.

GENERALIZED MINIMAX PRINCIPLE.—*Under the above definitions and postulates the critical extremals of type* k *of the functional* J *exist and their counts satisfy the inequalities* (1), (2), (3), (4).

4. *Applications to the Calculus of Variations.*—It has long been recognized that in the Calculus of Variations we are dealing with a functional J defined over a function space Ω. For example, in the simple case in which we seek to find extremals of an integral I in a class of arcs joining two fixed points, each arc of the class is considered as a point of Ω and the integral I as the functional J.

In view of the results of the last section the problem of finding extremals of type k has been reduced to that of suitably defining k-chains and deformations D_k on Ω. The deformations used by Birkhoff and Morse in the case of simple integrals involve the use of broken extremals, and form a sub-class of the admissible deformations D_k.

The minimax principle as formulated above is one of extraordinary generality, applying, for instance, to multiple integrals as well as simple integrals. A very simple example in double integrals is the following one. Consider the region between two concentric spheres S_1 and S_2. Let M be a three-dimensional analytic Riemannian manifold homeomorphic with the region obtained by identifying points of S_1 and points of S_2 which lie on same radii. Consider the class Ω of surfaces on M which are homeomorphic with the image of S_1 and have a finite area less than a constant C, sufficiently large. Such a surface is thought of as a point of Ω. The connectivities of Ω are clearly $R_0 = 1$, $R_1 = 1$, $R_j = 0 (j > 1)$. Hence if the functional $J(P)$ is defined to be the area of the point (surface) P it is clear that there must exist at least one minimal surface of least area and one minimal (minimax) surface of type one, provided, of course, that the existence of a suitable class of deformations be established.

A full treatment and applications of the "generalized minimax principle" as well as a study of the "natural isoperimetric conditions" of the immediately following Note will be given by us in a forthcoming article in the Duke Mathematical Journal.

[1] *Dynamical Systems with Two Degrees of Freedom, Trans. Amer. Math. Soc.*, **18** (1917).

[2] For references to Morse's work of 1925 and later see his book *The Calculus of Variations in the Large*, Colloquium *Pub. Amer. Math. Soc.*, **18** (1934).

[3] The theorem of Birkhoff (*Dynamical Systems*, p. 135) that any surface homeomorphic with a hypersphere has at least one closed geodesic depends for its proof upon a simple case of this extension.

[4] It is understood that every sub-k-cycle of a k-cycle in $\mathfrak{N}_k$ is also in $\mathfrak{N}_k$, and that homologous k-cycle in $\mathfrak{N}_k$ having the same critical k-sets are to be regarded as identical. A similar remark holds for k-chains in $\mathfrak{M}_k$.

NATURAL ISOPERIMETRIC CONDITIONS IN THE CALCULUS OF VARIATIONS

BY G. D. BIRKHOFF AND M. R. HESTENES

DEPARTMENT OF MATHEMATICS, HARVARD UNIVERSITY

Communicated January 12, 1935

1. *Introduction.*—In the present note we formulate the following principle:[1]

Every extremal of a positively regular integral J satisfying a prescribed set of end conditions affords a minimum to J, relative to neighboring admissible arcs satisfying these end conditions and a suitably chosen set of isoperimetric conditions which have the further property that every extremal of J satisfying the prescribed end conditions also satisfies these (natural) isoperimetric conditions.

Thus we see that under this principle every extremal can be basically characterized by the minimum number of natural isoperimetric conditions which are needed to reduce it to one of minimum type.

The chief purpose of this note is to define such "natural isoperimetric conditions" for the fixed end-point problem in the calculus of variations and to give some illustrations of its numerous applications. The results described in this paper can be extended readily to variable end-point problems and to problems concerned with closed extremals, reversible or irreversible.

One of the special features of the use of natural isoperimetric conditions is that it enables one to obtain easily and naturally the usual oscillation, separation and comparison theorems by classical methods of the Calculus of Variations instead of by the use of the accessory boundary value problems, as has been done heretofore. In fact, the use of artificial orthogonality conditions becomes unnecessary since these arose only because the natural isoperimetric conditions had not been used.

2. *Natural Isoperimetric Conditions.*—Consider a class of arcs joining two fixed points 1 and 2 in $(x^1, \ldots, x^n)$ space on which the integral

$$J = \int^{t_2} f(x, \dot{x})dt$$

is well defined. Let $H(x, \dot{x})$ be a function satisfying the conditions

$$H(x, k\dot{x}) = H(x, \dot{x})(k > 0), \quad H(x_1, \dot{y}) = H(x_2, z)$$

for all $(\dot{y}) \neq (0)$ and $(\dot{z}) \neq 0$, and let $\varphi^i(x, \dot{x})$ be a solution of the equations

$$H_{\dot{x}^k} = \varphi^i f_{\dot{x}^i \dot{x}^k}.$$

The condition

$$K = \int_{t_1}^{t_2} \{f_{x^i}\varphi^i + (H_{x^k} - \varphi^i f_{\dot{x}^i x^k})\dot{x}^k\}dt = 0$$

will be called a *natural isoperimetric condition.* It is satisfied by every extremal of J joining the points 1 and 2. A simple and important case is the one in which $H = \varphi^i(x)f_{\dot{x}^i}$; in this case K takes the form

$$K = \int_{t_1}^{t_2} \{f_{x^i}\varphi^i + f_{\dot{x}^i}\varphi^i_{x^k}\dot{x}^k\}dt = 0. \tag{1}$$

We have the immediate theorems:

THEOREM 1. *An admissible arc joining the points* 1 *and* 2 *which has continuous first and second derivatives and satisfies all natural isoperimetric conditions is a solution of the Euler equations.*

THEOREM 2. *Let E_{12} be an extremal arc (not intersecting itself) whose end points are not conjugate and which satisfies the strengthened conditions of Weierstrass and Legendre. Let m be the sum of the orders of the conjugate points of the point* 1 *on E_{12}. Then there exists a set of m (and no fewer) natural isoperimetric conditions of the form* (1) *such that E_{12} affords a proper strong relative minimum to J in the class of arcs joining its end-points and satisfying these m conditions.*

Thus we see that every extremal of the integral J can be considered as one of minimum type in a suitably chosen natural isoperimetric problem.

3. *The Accessory Problem and the Accessory Boundary Value Problem.*— It is not likely that there exist always a set of natural isoperimetric conditions such that every isoperimetric extremal satisfying the end conditions is an extremal of the integral J. Nevertheless these do exist in certain non-parametric problems, such as the accessory problem associated with the second variation of an integral J in non-parametric form. Here we study an integral of the form

$$J_2(\eta) = \int_{x_1}^{x_2} 2\,\omega(x, \eta, \eta')dx$$

where 2ω is a quadratic form in η_i and η_i' and the functions $\eta_i(x)$ vanish

at x_1 and x_2. The natural isoperimetric condition analogous to (1) is of the form

$$J_2(\zeta, \eta) = \int_{x_1}^{x_2} \{\zeta_i \omega_{\eta_i} + \zeta_i' \omega_{\eta' i}\} dx = 0 \qquad (2)$$

where $\zeta_i(x)$ vanishes at x_1 and x_2. If there are m conjugate points of x_1 between x_1 and x_2 and if the strengthened Legendre condition holds, then by the adjunction of m (and no fewer) suitable natural isoperimetric conditions the arc $(\eta) \equiv (0)$ becomes a minimizing arc relative to these conditions. This result is true even if the point x_2 is conjugate to x_1 and is obtained by the use of classical methods in the Calculus of Variations. Thus we can count the number of conjugate points of x_1 between x_1 and x_2 by counting the minimum number of isoperimetric conditions necessary to reduce the arc $(\eta) \equiv (0)$ to a minimizing arc.

Heretofore in the study of the accessory boundary value problem

$$(d/dx)\omega_{\eta_i}' - \omega_{\eta_i} + \sigma\eta_i = 0 \qquad \eta_i(x_1) = \eta_i(x_2) = 0$$

it has been a common procedure to adjoin isoperimetric conditions of an artificial type so as to make the arc $(\eta) \equiv (0)$ a minimizing arc. The number of conditions needed is then equal to the number of negative characteristic roots $\sigma < 0$, each counted a number of times equal to its multiplicity. These artificial conditions can be replaced by the natural isoperimetric conditions (2) and so we obtain a new proof of the following result due in the general case to Morse:

THEOREM 3. *The number of characteristic roots $\sigma \leqq 0$ is equal to the sum of the orders of the conjugate points of x_1 on x_1x_2.*

4. *Oscillation, Separation and Comparison Theorems.*—Since natural isoperimetric conditions afford a method of counting the number of conjugate points on an interval it is clear that they lead to oscillation, separation and comparison theorems as well. A typical oscillation theorem of apparently new type is the following:

THEOREM 4. *There exist (at least) m conjugate points of x_1 between x_1 and x_2 if and only if there exist a set of m admissible variations $\eta_{i\alpha}(x)$ $(\alpha = 1, \ldots, m)$ of the original problem vanishing at x_1 and x_2 for which*

$$J_2(\eta_\alpha\pi_\alpha) = J_2(\eta_\alpha, \eta_\beta)\pi_\alpha\pi_\beta < 0 \qquad (\alpha,\beta = 1, \ldots, m)$$

for every set of constants $(\pi) \neq (0)$ where $J_2(\zeta, \eta)$ is the integral (2).

The key point of the proof of this theorem is that $J_2(\eta_\alpha, \eta) = 0$ are a set of natural isoperimetric conditions.

5. *Problems Admitting a Group of Automorphisms.*—Frequently an extremal E_{12} has associated with it a group of automorphisms; in such a case there is in general a least sub-arc E_{13} of E_{12} with the property that every point on E_{12} is the image of a point on E_{13} under this group. If we compare

the value of the integral J on E_{12} with neighboring arcs under the same group and satisfying the prescribed end conditions, then it is clear that the number of natural isoperimetric conditions needed to reduce the arc E_{12} to one of minimum type in this class depends only on the nature of the sub-arc E_{13} and the group. In many cases if there are l conjugate sub-arcs the indices and number of natural isoperimetric conditions for a fundamental sub-arc at most equal those for the whole arc (regardless of the group) divided by l.

For example in the restricted problem of three bodies (see the next section), the group is given by $(x, y) \longrightarrow (\pm x, \pm y)$, and l is 2.

5. *An Application of Natural Isoperimetric Conditions.*—Natural isoperimetric conditions not only give rise to a simple characterization of extremals but also give us information concerning their location. A good example of this is afforded by the restricted problem of three bodies discussed by G. W. Hill. Here we are concerned with the closed extremals of the integral

$$J = \int_C \{xy' - yx' + (2\Omega - c)^{1/2}(x'^2 + y'^2)^{1/2}\}dt$$

where $2\Omega = 3x^2 + 2(x^2 + y^2)^{-1/2}$. A natural isoperimetric condition in this case is the condition

$$K = \int_c (y\Omega_x - x\Omega_y)(2\Omega - c)^{-1/2}(x'^2 + y'^2)^{1/2}dt = 0$$

as one readily verifies. It is clear that on every closed extremal E the expression

$$y\Omega_x - x\Omega_y = 3xy$$

must change sign. Hence every closed extremal whatsoever must cross one of the coördinate axes.

[1] See a paper by Birkhoff *Sur le problème restreint des trois corps* about to appear in the Pisa ***Annali,*** in which the possibility of natural isoperimetric conditions is alluded to.

SUBSUMPTION OF THE THEORY OF BOOLEAN ALGEBRAS UNDER THE THEORY OF RINGS

BY M. H. STONE

DIVISION OF MATHEMATICS, HARVARD UNIVERSITY

Communicated December 19, 1934

In a previous communication,[1] we have sketched an analogy between Boolean algebras and abstract rings, with particular reference to the theory of ideals and ideal arithmetic. In the present note we shall show that Boolean algebras are actually special instances, rather than analogs, of the general algebraic systems known as rings. In establishing this result we must choose the fundamental operations in a Boolean algebra in an appropriate manner, indicated below. The algebraic theory developed in the previous communication is thus a particular instance of the theory of rings and can be deduced in part from the known theorems concerning rings. These facts were discovered just as the detailed exposition of our independent theory of Boolean algebras was on the point of completion; and they now compel a radical revision which will necessarily delay publication of the complete theory.

If A is a Boolean algebra, with zero element 0 and unit e, we consider it as a system with double composition in which the fundamental operations are differentiation and multiplication. We denote the operation of differentiation by the symbol Δ and call the element $a\Delta b$ the symmetric difference of a and b. Since the operations of differentiation and addition are uniquely determined in terms of one another and the operation of multiplication by the relations $(a\Delta b) + ab = a + b$, $(a\Delta b)(ab) = 0$ and $a + b = (a\Delta b)\,\Delta ab$, respectively, our choice of fundamental operations does not affect the intrinsic nature of A as an algebraic system. If we express the symmetric difference in terms of addition and negation by the equation $a\Delta b = ab' + a'b$, we find without difficulty that the system A has the following properties:[2]

(1) $a\,\Delta b = b\,\Delta a$; (2) $a\,\Delta(b\,\Delta c) = (a\,\Delta b)\,\Delta c$; (3) the equation $x\,\Delta b = a$ has a (necessarily unique) solution $x = a\,\Delta b$; (4) $ab = ba$; (5) $a(bc) = (ab)c$; (6) $a(b\,\Delta c) = (ab)\ \Delta\ (ac)$; (7) $a\Delta 0 = a$; (8) $a\,\Delta a = 0$; (9) $aa = a$; (10) $ea = a$.

Properties (1)–(6) inclusive show that A is a commutative ring,[3] the operation Δ being the operation of ring addition usually denoted by $+$. Property (7) shows that the Boolean element 0 is the zero of the ring; and property (10) shows that the Boolean element e is a unit in the ring.

On the other hand, if R is a commutative ring which possesses a unit e and which has the special properties (8) and (9)—in other words, if R is an

abstract system with all ten properties (property (7) follows from (1)–(3))—we consider it as a system with double composition in which the fundamental operations are multiplication and an operation $+$ defined by the equation $a + b = (a \Delta b) \Delta ab$. If we introduce also the operation $'$ defined by the equation $a' = a \Delta e$, we easily deduce the following properties:

$(\alpha)\ a + b = b + a;\ (\beta)\ a + (b + c) = (a + b) + c;\ (\gamma)\ ab = (a' + b')';$
$(\delta)\ ab + ab' = a.$

These properties show that R is a Boolean algebra in accordance with a recent paper of Huntington;[4] and the elements 0 and e in R are easily identified as the Boolean zero and unit, respectively.

In the remainder of the note, we revert to the usual notation for ring addition, writing $+$ in place of Δ in (1)–(10); and we shall drop the requirement of the existence of a unit. Thus we shall consider an abstract commutative ring R with the special properties (8) and (9), (8) now being written as $a + a = 0$, or, equivalently, $a = -a$. We omit (7) as a consequence of (1)–(3). We may also, according to a suggestion of J. v Neumann, omit (8) as a consequence of the other properties. Thus we consider a commutative ring all of whose elements are idempotent. By specializing to such a ring the well-known theorems concerning the homomorphisms of rings,[3] we can immediately obtain Theorem I of our previous communication.[1]

We now show that Krull's theory of ideal arithmetic in rings with absolute values[5] is applicable to R, at least in principle. To this end, we determine all real-valued functions $|a|$ with argument a in R which assume two or more distinct values and which have the property (11) $|ab| = |a| \cdot |b|$. By (9) and (11), it is clear that $|a| = |a|^2$ and hence that the value of $|a|$ is either 0 or 1. Using the relation $0 = 0a$ and choosing a so that $|a| = 0$, we find that $|0| = |0a| = |0||a| = 0$. Since $(a + b)ab = 0$, it is clear that $|a + b||a||b| = |0| = 0$ and hence that the relations $|a| = |b| = 1$ imply $|a + b| = 0$. By a simple combinatorial analysis and the relation $b = -b$, we now find that

(12) $|a + b| \leqq \max(|a|, |b|)$; (13) $|a - b| = 0$ if and only if $|a| = |b|$.

The relations (11) and (12) are precisely those upon which Krull bases his theory. From (13) we can show without difficulty that the classes $\mathfrak{A}$ and $\mathfrak{B}$ specified by the respective relations $|a| = 0$, $|a| = 1$ have the following properties: $\mathfrak{A}$ is an ideal in R; $\mathfrak{A}$ and $\mathfrak{B}$ are both residual classes (mod $\mathfrak{A}$) and they are the only such classes; and hence the ideal $\mathfrak{A}$ is both prime and divisorless. Every ideal $\mathfrak{A}$ with these properties determines an absolute value $|a|$ in R. Once the existence of prime ideals in R is established, Krull's results serve to establish Theorem III of our previous communica-

tion; and they yield also the first part of Theorem IV_1, up to the words "with the following properties."

We have thus shown that the theory of Boolean algebras, with particular reference to ideal-theoretic and arithmetic properties, is a special case of the theory of rings. We can say further that the theory of those algebraic systems known as distributive lattices[6] can be considered as a part of the theory of rings: for an unpublished result due to Mr. Holbrook MacNeille shows that every distributive lattice can be imbedded by a purely algebraic construction in a Boolean algebra.

An interesting consequence of the theory developed in this note is that it is now possible to describe the mathematical theory of probability entirely in the language of the theory of rings. It is the theory[7] of functions $p(a)$ which are defined over a ring R of the special type described above, which assume values in an ordered ring P, and which have the special properties $p(a) \geqq 0$, $p(a + b) = p(a) + p(b)$ when $ab = 0$. The elements of R are events, and $p(a)$ is the relative probability of the event a. In general, it is not necessary to assume that P is a commutative ring or that the ordering in P is archimedean. If we assume that P is a commutative ring with archimedean order, then P is isomorphic to a subring of the real number system. We point out that the construction of the absolute value $|\ a\ |$ discussed above provides a simple illustration of the present theory, since $|\ a\ | \geqq 0$ and since $ab = 0$ implies $|\ a + b\ | = |\ a\ | + |\ b\ |$.

[1] *Proc. Nat. Acad. Sci.*, **20**, 197–202 (1934).

[2] All of these properties have been known for some time. See, for example, Daniell, *Bull. Am. Math. Soc.*, **23**, 446–458(1916); B. A. Bernstein, *Trans. Am. Math. Soc.*, **26**, 171–175 (1924), and **28**, 654–657 (1926).

[3] See B. L. van der Waerden, *Moderne Algebra*, **1**, Chapter 3 (Berlin, 1930).

[4] Huntington, *Trans. Am. Math. Soc.*, **35**, 274–304 and 557–558, especially the latter, (1933).

[5] See Ore, *Bull. Am. Math. Soc.*, **39**, 728–745 (1934), especially 742–745; and Krull, *Math. Zeit.*, **29**, 42–54 (1929), *Math. Zeit.*, **31**, 526–557 (1930), and *J. für Math.*, **167**, 157–196 (1932).

[6] See Garrett Birkhoff, *Proc. Camb. Phil. Soc.*, **29**, 441–464, especially 442 and 453 (1933).

[7] See Kolmogoroff, *Grundbegriffe der Wahrscheinlichkeitsrechnung*, 2 (Berlin, 1933), and B. L. van der Waerden, *Moderne Algebra*, **1**, Chapter 10, the latter for the theory of ordered rings and fields (Berlin, 1930).

LOCALLY COMPACT RINGS

BY N. JACOBSON AND O. TAUSSKY*

PRINCETON UNIVERSITY AND BRYN MAWR COLLEGE

Communicated January 10, 1935

1. L. Pontrjagin[1] has given a topological characterization of the fields of real numbers, complex numbers and quaternions. Recently[2] he described the structure of locally compact and connected abelian groups satisfying the second axiom of countability (separable). As every field and every ring (commutative or not) is an abelian group with respect to addition, it is possible to derive the previous results of Pontrjagin from his theorems on groups and it is possible to obtain by this method far more general results concerning topological rings.

E. R. van Kampen[3] extended Pontrjagin's main theorems on groups to the non-connected case. The following is the Pontrjagin-van Kampen theorem which is fundamental for the present paper:

Any locally compact and separable abelian group is the direct sum of a vector group N and a group C in which the component C_1 of the 0-element is compact.

From this it follows that C_1 is the maximal compact and connected subgroup of the whole group.

We are concerned with topological rings, i.e., having an infinite number of elements and for which $a + b$ and $a \cdot b$ are continuous functions of both variables.

By means of the above theorem we shall extend Pontrjagin's theorem on fields to the non-connected and non-associative case:[4]

THEOREM I. *A locally compact and separable (not necessarily associative or commutative) field F is either a hypercomplex system over the real field or is totally disconnected.*

2. LEMMA 1. *A field satisfying the first axiom of countability cannot be compact.*

If the field F were compact it would contain a sequence $a_n \longrightarrow 0$. Then the sequence a_n^{-1} diverges. For if $a_{n_j}^{-1}$ is a sub-sequence converging to a limit a, then $a_{n_j}a_{n_j}^{-1} = 1 \longrightarrow 0$, which is impossible.

LEMMA 2. *Let N be a topological ring which is—considered as a group with respect to addition—continuously isomorphic to an n-dimensional real vector group. Then N is a hypercomplex system over the field of real numbers.*

From the isomorphism and continuity it follows that N contains n elements such that every element of N is a linear form with real coefficients in these elements. It is merely necessary to show that the automorphism $x \longrightarrow \alpha x$ where α is a real number, commutes with multiplication: $x(\alpha y) = \alpha(xy) = (\alpha x)y$. This holds for integral values of α by the distributive

law. Since there are no elements of finite order in N, it holds for rational values. By continuity it holds for all real α.

3. We now prove Theorem I. As F is a locally compact and separable abelian group when regarded additively, it is a direct sum $F = N + C$ where N is a vector group and C is a group in which the component C_1 of 0 is compact. Now C_1 is an ideal: If x is any element of F the set xC_1 is a compact and connected sub-group and hence $xC_1 \subset C_1$; in the same way it follows that $C_1x \subset C_1$. Since F is a field it contains only the 0-ideal and the 1-ideal. Thus either $C_1 = 0$ or $C_1 = F$. But by Lemma 1, $C_1 = F$ is impossible and so $C_1 = 0$.

Now $F = N + C$ where N is a vector group and C a group in which the component of 0 is 0 itself, i.e., C is totally disconnected. We prove now that either $F = N$ or $F = C$. N is an ideal. For in the group F, N is the component of 0 and xN is a connected set containing 0. Hence $xN \subset N$ and similarly $Nx \subset N$. Since N is an ideal either $F = N$ and the field is, according to Lemma 2, a real hypercomplex system; or, $F = C$ and the field is totally disconnected.

If F is associative we obtain from Theorem I and the Frobenius theorem the

THEOREM II. *A locally compact and separable associative field is isomorphic to the field of real numbers, of complex numbers or of quaternions, or is totally disconnected.*

4. THEOREM III. *A locally compact, separable and connected (not necessarily associative or commutative) ring R which contains no absolute zero-divisor* (i.e., *an element c such that $cR = Rc = 0$) is a hypercomplex system over the real field.*

The assumption that R contains no absolute zero-divisor holds, for instance, if R has an identity or if R has no nilpotent ideal.

The original Pontrjagin result on connected groups shows that $R = N + C$ where N is a real vector group and C is compact and connected. Therefore it follows as before that C is an ideal. We proceed to show that $C = 0$.

LEMMA 3. *C cannot have all its elements of finite order.*

Let χ be a character of C. If c is of order m, $m\chi(c) = \chi(mc) = 0$ and $\chi(c)$ is a rational point on the circle. χ has only rational values. Since χ is continuous and C connected $\chi(c) = 0$ for all c. This contradicts the fact that non-zero characters exist for C.[5]

LEMMA 4. *$C = mC$ where m is a positive integer and mC is the set of elements mc, c in C.*

mC is closed; hence C is continuously homomorphic with the quotient group $C - mC$. The latter is compact and connected and has only elements of finite order. By Lemma 3, $C - mC = 0$, or $C = mC$.

LEMMA 5. *If C' is the closure of the group generated by the elements of infinite order of C, then $C' = C$.*

This follows immediately from the fact that $C - C'$ has only elements of finite order.

Let us now prove Theorem III. We shall show that $RC = CR = 0$ and from the assumption that R contains no absolute zero-divisor will follow that $C = 0$.

(1) Let $x \epsilon N$ and $xC = C'$. Then $\left(\frac{1}{m}x\right)C = \left(\frac{1}{m}x\right)(mC) = xC = C'$ (m a positive integer). If $u \epsilon C'$ for every m we have a c_m such that $\left(\frac{1}{m}x\right)c_m = u$. A sub-sequence of $\{c_m\}$ converges to a limit c and hence $u = 0 \cdot c = 0$ and $C' = 0$.

(2) Let $x \epsilon C$ and have infinite order. For every integral m the relation $(mx)C = x(mC) = xC = C'$ holds. There exists a sequence of integers m_j such that $m_j x \longrightarrow 0$. It follows then as in (1) that $xC = 0$. By Lemma 5 we have for *every* $x \epsilon C$, $xC = 0$.

5. In the case of non-connected rings we may apply the above arguments to C_1 the compact component of 0 in place of C. We may show that $C_1 C_1 \neq 0$ and $C_1 N = N C_1 = 0$ (not necessarily $C C_1$ or $C_1 C$, however). If we assume, for example, that the ring has no nilpotent ideal, then $C_1 = 0$ and $R = N + C$ where N is an ideal and a real hypercomplex system. Again, if we suppose that R is *simple*, i.e., has only the 0- and 1-ideals then R is either a real hypercomplex system or totally disconnected.

* Alfred Jarrow Scientific Research Fellow.

[1] L. Pontrjagin, "Über stetige algebraische Körper," *Ann. Math.*, **33**, 163–174 (1932).

[2] L. Pontrjagin, "The Theory of Topological Commutative Groups," *Ibid.*, **35**, 361–388 (1934).

[3] E. R. van Kampen, "Locally Compact Abelian Groups," *Proc. Nat. Acad. Sci.*, **20**, 434–436 (1934).

[4] D. van Dantzig considers non-connected fields in "Topologische Algebra I," *Math. Ann.*, **107**, 589 (1932). An example of a connected non-associative field is the field of the Cayley numbers.

[5] This has been proved by Pontrjagin by means of the Peter-Weyl theory of representations and the Haar group measure, l. c. in footnote 2.

ELECTRICAL RESISTANCES AND VOLUME CHANGES UP TO 20,000 KG./CM.²

BY P. W. BRIDGMAN

RESEARCH LABORATORY OF PHYSICS, HARVARD UNIVERSITY

Communicated December 21, 1934

This note presents a brief summary of several phenomena at pressures up to 20,000 kg./cm.² which will be described in full detail in Proceedings American Academy of Arts and Sciences. 20,000 kg./cm.² represents a considerable extension of range of my previous measurements, which usually reached to 12,000. I had formerly in one instance made accurate measurements up to 20,000, but the apparatus did not survive a single application of pressure, so that the expense of making similar measurements of other phenomena would have been prohibitive at that time. In the last few years, however, new grades of steel of much superior strength have been developed, so that the life of the apparatus subject to 20,000 is now long enough to justify the serious attempt to measure in the wider range such phenomena as experience in the 12,000 range have shown to be of probable special significance. As subjects for these first measurements over the extended range were chosen the electrical resistance of black phosphorus and tellurium, both of which show very large effects up to 12,000, and the pressure-volume-temperature relations of the alkali metals lithium, sodium and potassium, with especial emphasis on the thermal expansion.

A number of modifications in technique were introduced, which are fully described in the detailed paper. In spite of all these modifications, measurements in the extended field remain much more difficult than in the original range. The essential difficulty is the short life of the apparatus, and the fact that an explosion may involve not only the loss of the pressure vessel itself but also of the apparatus within the vessel. In order to obtain the data of this note, five different pressure vessels had to be used; contrasted with this is the fact that it is now several years since a pressure vessel used only up to 12,000 has broken.

Perhaps the most fundamental question of technique in the extension of range is the validity of the pressure measurements. The pressure gauge used up to 12,000 has been the manganin resistance gauge, in which pressure is measured by the change of resistance of a coil of manganin wire. An initial calibration of this gauge against an absolute gauge up to 12,000 showed the relation between change of resistance and pressure to the linear within 1/10% over this range. The limit of the absolute gauge is about 12,000, and pressures between 12,000 and 20,000 are measured by a linear extrapolation of the manganin gauge. In order to justify this

extrapolation as far as possible, a preliminary study was made of the effect of pressure on the resistance of the pure metals iron, silver and gold. These were compared with the manganin gauge over the entire range. The resistance of the pure metals is not linear in the pressure, but is approximately parabolic. The procedure was to determine the constants of the two-power expression from the measurements up to 12,000 only, and then to use the formulas so determined to extrapolate the resistances up to 20,000, pressure being assumed accurately given by the manganin gauge. The result was almost perfect agreement, from which the highly probable conclusion is to be drawn that the resistance of all four metals, manganin, iron, silver and gold exhibits no change of trend between 12,000 and 20,000, and in particular that the resistance of manganin remains linear over this range. I believe that the manganin gauge is good up to 20,000 within two or three tenths of one per cent.

The results of the measurements on the resistance of black phosphorus are shown in table 1. The effect of pressure is so large that it is more convenient to give the logarithm of the resistance rather than the resistance itself. It will be seen that the resistance at 20,000 has dropped to 0.0069 of its value at atmospheric pressure. This large decrease does not continue indefinitely, however, for the first differences for pressure intervals of 20,000 kg./cm.^{2} plotted against pressure show a very rapid drop beyond 10,000. At the upper end of the pressure range the drop is so rapid that a short extrapolation indicates a vanishing of the first difference at about 23,000 kg./cm.^{2}. That is, there is in all probability a minimum in the resistance at 23,000, beyond this the resistance increasing with increasing pressure. This is similar to the resistance of the alkali metals. Table 1 shows also the temperature coefficient; this reverses sign at 12,000, becoming positive, like the pure metals, above 12,000, and at 20,000 having

TABLE 1

RESISTANCE OF BLACK PHOSPHORUS

PRESSURE Kg./cm.^{2}	Log_{10} R(p,τ)/R(0.30°) 30°	75°	TEMPERATURE COEFFICIENT
0	0.000	9.871	−.0077
2000	$\bar{9}$.787	9.655	79
4000	9.566	9.441	74
6000	9.332	9.223	63
8000	9.083	9.001	46
10000	8.820	8.779	22
12000	8.554	8.554	00
14000	8.298	8.325	+.0014
16000	8.078	8.118	21
18000	7.926	7.971	24
20000	7.838	7.883	25

a numerical value about $^3/_4$ that characteristic of most pure metals (0.0025 against 0.0033). The general effect of pressure on the resistance of black phosphorus is therefore to make it approach that of the pure metals in behavior.

In table 2 are given results for two different orientations of single crystal tellurium, from which the results for any other orientation may be cal-

TABLE 2

RESISTANCE OF TELLURIUM

PRESSURE Kg./cm.²	LENGTH 23½° WITH AXIS $\log_{10} R(p,\tau)/R(0.30°)$ 30°	75°	TEMPERATURE COEFFICIENT	LENGTH 86° WITH AXIS $\log_{10} R(p,\tau)/(R(0.30°)$ 30°	75°	TEMPERATURE COEFFICIENT
0	0.000	$\bar{9}$.704	−.0217	0.000	$\bar{9}$.697	−.0224
2000	$\bar{9}$.709	9.405	226	$\bar{9}$.691	9.377	236
4000	9.430	9.145	206	9.395	9.094	222
6000	9.165	8.910	178	9.119	8.841	199
8000	8.921	8.693	154	8.865	8.615	178
10000	8.705	8.496	137	8.634	8.410	150
12000	8.510	8.318	124	8.423	8.227	128
14000	8.332	8.161	108	8.230	8.061	106
16000	8.170	8.022	090	8.053	7.908	088
18000	8.025	7.898	75	7.891	7.768	73
20000	7.897	7.789	63	7.752	7.644	63

culated. The tellurium was the identical material on which previous measurements have been made between liquid air temperatures and 95°C. over a narrower pressure range.[1] In general, the results are dependent on the orientation to only a minor degree. Qualitatively the results are similar to those for black phosphorus. The absolute magnitude of the effect of pressure is about the same, the decrease of resistance under 20,000 being very large. There is also a very large decrease of the pressure coefficient with pressure. If first differences are plotted against pressure a decrease at 20,000 to less than 40% of the initial value will be found. Extrapolation indicates a probable minimum in the resistance at about 30,000. The temperature coefficient of tellurium is initially large and negative; the absolute value decreases at 20,000 to less than one third its initial value. Extrapolation suggests a reversal of sign of the temperature coefficient at higher pressures.

The general effect of pressure on the resistance of the two semi-conductors black phosphorus and tellurium is therefore to make them approach the behavior of the metals. Another semi-conductor, Cu_2S, was also measured to 20,000. This was quite different in behavior. The effect of pressure on resistance was comparatively small, the decrease at 20,000 being about 11%. The situation has elements of complexity not shown by the others; there is a very marked discontinuity in the derivative between 2000 and 3000, and at 75° there are irreversible effects, although at 30° the relations

are perfectly reversible. It is therefore evident that the effect of pressure on the resistance of semi-conductors may be of more than one simple type.

TABLE 3

PRESSURE-VOLUME-TEMPERATURE RELATIONS OF THREE ALKALI METALS

	LITHIUM		SODIUM		POTASSIUM	
PRESSURE Kg./cm.²	$\Delta V/V_0$ at 0° C.	MEAN LINEAR EXPANSION 0°–95°	$\Delta V/V_0$ at 0° C.	MEAN LINEAR EXPANSION 0°–95°	$\Delta V/V_0$ at 0° C.	MEAN LINEAR EXPANSION 0°–95°
0		(.000056$_0$)		(.000071)		(.000083)
2000	.0164	51_5	.0295	62_2	.0571	67_0
4000	.0320	47_3	.0552	55_8	.1002	53_8
6000	.0466	43_4	.0779	49_5	.1347	42_7
8000	.0606	40_0	.0981	45_0	.1640	33_8
10000	.0739	37_0	.1165	40_8	.1890	27_0
12000	.0866	34_8	.1332	37_0	.2108	22_0
14000	.0984	32_8	.1488	33_7	.2300	18_1
16000	.1094	30_6	.1632	30_0	.2472	15_5
18000	.1198	28_8	.1767	26_6	.2626	13_8
20000	.1296	27_3	.1894	23_2	.2764	12_5

In table 3 are collected results on the pressure-volume-temperature relations of lithium, sodium and potassium. Measurements of the volume as a function of pressure up to 12,000 have been previously published. On comparing the present results with the previous ones, a serious error came to light in the previous work. This error was in the reduction of $\Delta l/l_0$ to $\Delta V/V_0$, the second order correction being applied with the wrong sign. The result was that the previous changes of volume are too large; the percentage effect on the change of volume depends on the absolute magnitude of the change and is therefore largest at the high pressures and vanishes at low pressures. The same error affects also all my other published values or compressibility up to 1924, but is comparatively unimportant in most of these cases because the volume changes are so much smaller. I intend to publish the corrected values in detail in Proceedings American Academy. The present values for ΔV agree rather well with the former values after making the proper second order correction, thus checking the experimental methods, which were somewhat different in the two cases.

If plots are made of volume and of compressibility against pressure, perfectly smooth curves will be obtained; compressibility decreases smoothly with increasing pressure over the entire range, and the compressibility increases smoothly from lithium to sodium to potassium over the entire range.

The particular feature of these new measurements is the determination of the thermal expansion, which is of particular interest for these metals; previous attempts to determine it had not been very successful. The

thermal expansions as now determined in the first place decrease with increasing pressure by a larger factor than does the compressibility. The reverse has hitherto been found to be the case, but previous measurements have been mostly confined to liquids, in which there is a very rapid decrease of compressibility at low pressure. Whether the more rapid decrease of thermal expansion is characteristic of metals in general or is peculiar to these alkali metals can be told only by further experiments.

In the second place the thermal expansions show the very notable feature that there is an inversion of order; at low pressures potassium is the most expansible and lithium the least, but at 20,000 lithium is most expansible and potassium least. A similar inversion of order has previously been found in the melting points of sodium and potassium,[2] and extrapolation indicated a complete inversion of order of melting point of all the alkalies at sufficiently high pressures. The two inversions are doubtless intimately related. They suggest a rather drastic change in the metallic mechanism at high pressures. At low pressures the situation is controlled by the single valence electrons, which are more and more loosely attached with increasing weight of the alkali metals, so that lithium is the "hardest" and caesium the "softest" of the alkalies. But with increasing pressure the valence electron becomes absorbed in the structure, as indicated, for example, by very recent theoretical studies of the change of electrical conductivity with pressure,[3] so that at high pressure the situation is controlled by the inner shells, and we have increasing melting point with increasing atomic weight, such as is shown in the Fe, Pd, Pt series.

[1] P. W. Bridgman, *Proc. Amer. Acad.*, **68**, 114 (1933).
[2] P. W. Bridgman, *Phys. Rev.*, **3**, 153 (1914).
[3] N. H. Frank, forthcoming paper in *Phys. Rev.*

SEPARATION OF ROTATIONAL COÖRDINATES FROM THE SCHRÖDINGER EQUATION FOR N PARTICLES

By J. O. Hirschfelder and E. Wigner

Palmer Physical Laboratory, Princeton, N. J.

Communicated November 9, 1934

1. *Introduction.*—In quantum mechanics, as well as in classical mechanics, the number of variables involved in the equations of motion may be reduced by utilizing the constants of motion of the system. For a set of N particles moving in a field-free space there are six integrals if N is greater than two. These correspond to the laws of conservation of linear and of angular momentum. It is the purpose of this paper to elimi-

nate the coördinates of rotation and of translation of the total system from the Schrödinger equation and to obtain a new set of differential equations involving only $3N$-6 variables.[1]

The separation of the translation coördinates is simple. The total energy may be decomposed into the sum of the energy due to the motion of the center of mass and to the motion relative to the center of mass. The energy of the motion about the center of mass is obtained by subtracting the operator corresponding to the energy of translation of the center of mass from the total energy operator

$$H\psi = -\frac{h^2}{2}\sum_{n=1}^{N}\sum_{k=1}^{3}\frac{1}{m_n}\frac{\partial^2\psi}{\partial x_{nk}^2} + \frac{h^2}{2M}\sum_{k=1}^{3}\left(\sum_{n=1}^{N}\frac{\partial}{\partial x_{nk}}\right)^2\psi + V\psi = E\psi, \quad (1)$$

where M is the total mass of the system.[1] It may be shown that if ψ is an eigenfunction of H and U is a function of the coördinates of the center of mass, the product $U\psi$ is also an eigenfunction. Therefore equation (1) is really independent of the translational motion of the system. For our purposes, it will be convenient to consider (1) as the Eulerian equation of the variational problem:[2]

$$\delta\int\left\{\frac{h^2}{2}\sum_n\sum_k\left|\frac{\partial\psi}{\partial x_{nk}}\right|^2 - \frac{h^2}{2M}\sum_k\left|\sum_n\frac{\partial\psi}{\partial x_{nk}}\right|^2 + V\,|\,\psi\,|^2\right\}d\tau = 0, \quad (2)$$

and the condition $\int |\,\psi\,|^2\,d\tau = 1$.

The dependence of the eigenfunctions of H on the rotation of the total system is well known from group theory.[3] The eigenfunctions possess two quantum numbers l and μ, characteristic respectively of the total angular momentum lh and the Z component of angular momentum μh, and may be labeled $\psi_\mu^l(x_1, \ldots, z_N)$. If one defines for every configuration $x_1, \ldots, z_N$ a "standard position" $x_1', \ldots, z_N'$ which is the same for all configurations in which the relative position of the particles is the same, one can write

$$\psi_\mu^l(x_1 \ldots z_N) = \sum_{r=-l}^{l} D^{(l)}(R)_{\mu r}^{*}\, X_r(x_1, \ldots, z_N). \quad (3)$$

Here R is the rotation which brings the standard position $x_1', \ldots, z_N'$ into $x_1, \ldots, z_N$, the $D^{(l)}(R)_{\mu r}$ are the coefficients of the irreducible representations of the three dimensional rotation group. The

$$X_r(x_1, \ldots, z_N) = \psi_r(x_1', \ldots, z_N') \quad (3a)$$

are such functions of the $x_1, \ldots, z_N$ which depend on the relative positions of the particles only, since $x_1', \ldots, z_N'$ is the same set of numbers for all possible orientations of a relative configuration.

Once the standard position has been selected, (3) may be stubstituted into (2) and the integration performed over the rotational coördinates.

2. *Modified Schrödinger Equation in General Form.*—In order to substitute (3) into (2), it is necessary to evaluate the derivatives of the representation coefficients. Letting R and RS^{nk} be the rotation which brings the standard position into $x_{11}, \ldots, x_{N3}$ and $x_{11}, \ldots, x_{nk} + \epsilon, \ldots, x_{N3}$ (x_{nk} is the k-th coördinate of the n-th particle)

$$\frac{\partial D^{(l)}(R)_{\mu\nu}}{\partial x_{nk}} = \lim_{\epsilon=0} \frac{1}{\epsilon} [D^{(l)}(RS^{nk})_{\mu\nu} - D^{(l)}(R)_{\mu\nu}]. \tag{4}$$

Evidently S^{nk} is an infinitesimal rotation and can be decomposed into three infinitesimal rotations about the coördinate axes. The corresponding angles of rotation about the X, Y, Z axes may be denoted respectively by ϵS^{nk}_{32}, ϵS^{nk}_{13}, ϵS^{nk}_{21}. The S^{nk}_{ij} are functions of the coördinates, their forms depending on the choice of the standard position. If the standard position is independent of rotation and of translation of the total system, they satisfy the identities:

$$\sum_n S^{nk}_{ij} = 0 \qquad (i \neq j) \tag{5a}$$

$$\sum_n x_{na} S^{nb}_{ji} - x_{nb} S^{na}_{ji} = R_{ck} \quad \begin{matrix} a, b, c \text{ as well as } i, j, k \text{ form} \\ \text{cyclic permutations of } 1, 2, 3. \end{matrix} \tag{5b}$$

[(5b) will not be used further on.] Using (4) and the explicit form of the representation coefficients we obtain

$$\begin{aligned} \frac{\partial D^{(l)}(R)_{\mu\nu}}{\partial x_{nk}} &= i\nu S^{nk}_{21} D^{(l)}(R)_{\mu\nu} - \\ &\frac{1}{2}(S^{nk}_{32} - i S^{nk}_{13}) \sqrt{(l+\nu)(l-\nu+1)}\, D^{(l)}(R)_{\mu\nu-1} + \\ &\frac{1}{2}(S^{nk}_{32} + i S^{nk}_{13}) \sqrt{(l+\nu+1)(l-\nu)}\, D^{(l)}(R)_{\mu\nu+1} \end{aligned} \tag{6}$$

And therefore

$$\frac{\partial \psi^l_\mu}{\partial x_{nk}} = \sum_{-l}^{l} D^{(l)}(R)^*_{\mu\nu} \zeta^\nu_{nk} \tag{7a}$$

$$\begin{aligned} \zeta^\nu_{nk} = \frac{\partial X_\nu}{\partial x_{nk}} &- i\nu S^{nk}_{21} X_\nu - \frac{1}{2}(S^{nk}_{32} + iS^{nk}_{13}) \sqrt{(l+\nu+1)(l-\nu)}\, X_{\nu+1} \\ &+ \frac{1}{2}(S^{nk}_{32} - iS^{nk}_{13}) \sqrt{(l+\nu)(l-\nu+1)} X_{\nu-1}. \end{aligned} \tag{7b}$$

These derivatives may be substituted into the variational principle, (2),

$$\delta \int dR \int d\tau \sum_{\nu\nu'} D^{(l)}(R)^*_{\mu\nu} D^{(l)}(R)_{\mu\nu'} \left[\frac{h^2}{2} \sum_{nn'k} \left(\frac{\delta_{nn'}}{m_n} - \frac{1}{M} \right) \zeta^\nu_{nk} \zeta^{\nu'*}_{n'k} + V X_\nu X^*_{\nu'} \right] = 0 \tag{8a}$$

Using the orthogonality relations between the representation coefficients,

the integration may be performed over the rotational coördinates, R. One obtains:

$$\delta \int d\tau \sum_r \left[\frac{h^2}{2} \sum_{\substack{nn' \\ k}} \left(\frac{\delta_{nn'}}{m_n} - \frac{1}{M} \right) \zeta^r_{nk} \zeta^{r*}_{n'k} + V |X_r|^2 \right] = 0 \qquad (8b)$$

with the boundary condition

$$\int \sum_r |X_r|^2 \, d\tau = \text{Const.}$$

3. *The Standard Position.*—The next problem is that of defining the standard position for any configuration of the system. The best standard position should make the final equations as simple as possible without disturbing the symmetry between equivalent particles. We investigated several possibilities for the standard position and the most simple results were obtained if the center of gravity was in the origin of the coördinate system and the principal axes of inertia parallel to the coördinate axes. Therefore, we shall define R as the orthogonal matrix which brings:

$$T_{ik} = \sum_n m_n \left(x_{ni} - \sum_{n'} \frac{m_{n'} x_{n'i}}{M} \right) \left(x_{nk} - \sum_{n'} \frac{m_{n'} x_{n'k}}{M} \right) \qquad (9)$$

into the diagonal form

$$\sum_k T_{ik} R_{k\rho} = t_\rho R_{i\rho}. \qquad (10)$$

The $R_{i\rho}$ and the t_ρ are functions of the x-coördinates. It will be convenient to define the coördinates, $y_{n\rho}$, of the particles referred to the principal axes of inertia

$$y_{n\rho} = \sum_k R_{k\rho} \left(x_{nk} - \sum_{n'} \frac{m_{n'} x_{n'k}}{M} \right) \qquad (11)$$

They satisfy the identities:

$$\sum_n m_n \, y_{n\rho} \, y_{np'} = 0 \quad \rho \neq \rho' \qquad (12a)$$

$$\sum_n m_n \, y_{n\rho} = 0. \qquad (12b)$$

For the sake of definiteness we shall take: $t_1 \leq t_2 \leq t_3$.

The x_{nk} can be completely replaced by the three coördinates of the center of mass, the $R_{i\rho}$ (three independent variables) and the $y_{n\rho}$ ($3N$-6 independent variables). Of course, the wave function is defined only for such $y_{n\rho}$ which satisfy the six identities (12). However, in order to preserve the symmetry, it is advantageous to define the X_r also for such $y_{n\rho}$ which do not satisfy the identities by assuming that they depend only on the relative configuration in the y-space. These generalized wave

functions might still depend on the R's and the coördinates of the center of gravity, but they do not, since they are functions only of the relative configuration in x-space.

It is easy to calculate the $S^{nk}_{pp'}$ matrix by the Rayleigh-Schrödinger perturbation method. The R_{i1}, R_{i2}, R_{i3} are the three characteristic vectors of the T_{ij} for the original configuration $x_{11} \ldots x_{N3}$. If x_{nk} is changed by the small quantity ϵ, the $S^{nk}_{pp'}\epsilon$ are the coefficients of the original R_{ip} in the p'-th characteristic vector of the "perturbed" T_{ik}. The formulas of the Rayleigh-Schrödinger theory give

$$S^{nk}_{ij} = \frac{m_n}{t_j - t_i}\,(R_{kj}\,y_{ni} + R_{ki}\,y_{nj}) \tag{14a}$$

and

$$\frac{\partial t_p}{\partial x_{nk}} = 2m_n\,R_{kp}\,y_{np}. \tag{14b}$$

From (14) it follows that

$$\frac{\partial X_r}{\partial x_{nk}} = \sum_{n'p} R_{kp}\left(\delta_{nn'} - \frac{m_n}{M}\right)\frac{\partial X_r}{\partial y_{n'p}} - \sum_p S^{nk}_{p+2\,p+1}\,M_p X_r \tag{15a}$$

Where

$$M_c = \sum_n \left(y_{na}\frac{\partial}{\partial y_{nb}} - y_{nb}\frac{\partial}{\partial y_{na}}\right) \qquad \begin{matrix} a, b, c \text{ are cyclical per-} \\ \text{mutations of 1, 2, 3.} \end{matrix} \tag{15b}$$

Inserting this into the variational principle, (8), we obtain an explicit form:

$$\delta \sum_r \int \left\{\sum_{\substack{n'n \\ p}} \frac{h^2}{2}\left(\frac{\delta_{nn'}}{m_n} - \frac{1}{M}\right)\frac{\partial X_r}{\partial y_{np}}\frac{\partial X_r^*}{\partial y_{n'p}} + V|X_r|^2 + \right.$$
$$\left.\frac{h^2}{2}\sum_p \frac{t_{p+1} + t_{p+2}}{(t_{p+1} - t_{p+2})^2}\,|A_p|^2 - \frac{h^2}{2}\sum_p (A_p^* L_p X_r + A_p L_p X_r^*)\right\} d\tau. \tag{16}$$

Here

$$L_c = \frac{1}{t_a - t_b}\sum_n \left(y_{na}\frac{\partial}{\partial y_{nb}} + y_{nb}\frac{\partial}{\partial y_{na}}\right) \tag{16a}$$

$$A_1 = M_1 X_r + \frac{1}{2}\sqrt{(l+r+1)(l-r)}\,X_{r+1} - \frac{1}{2}\sqrt{(l+r)(l-r+1)}\,X_{r-1}$$

$$A_2 = M_2 X_r + \frac{i}{2}\sqrt{(l+r+1)(l-r)}\,X_{r+1} + \frac{i}{2}\sqrt{(l+r)(l-r+1)}\,X_{r-1}$$

$$A_3 = M_3 X_r + ir X_r. \tag{16b}$$

The Eulerian equations for the vanishing of the first variation of (16) are

$$EX_r = H_0 X_r + G'_{-2} X_{r-2} + G'_{-1} X_{r-1} + G'_0 X_r + G'_1 X_{r+1} + G'_2 X_{r+2} + F'_{-1} X_{r-1} + F'_0 X_r + F'_1 X_{r+1} \quad (17)$$

where

$$H_0 = -\sum_{n=1}^{N} \sum_{p=1}^{3} \frac{h^2}{2m_n} \frac{\partial^2}{\partial y_{np}^2} + V. \quad (17a)$$

$$\frac{2}{h^2} G'_{-2} = -\frac{1}{4} \sqrt{[l(l+1) - (r-1)^2]^2 - (r-1)^2} \left(\frac{t_2 + t_3}{(t_2 - t_3)^2} - \frac{t_1 + t_3}{(t_1 - t_3)^2} \right)$$

$$\frac{2}{h^2} G'_2 = -\frac{1}{4} \sqrt{[l(l+1) - (r+1)^2] - (r+1)^2} \left(\frac{t_2 + t_3}{(t_2 - t_3)^2} - \frac{t_1 + t_3}{(t_1 - t_3)^2} \right)$$

$$\frac{2}{h^2} G'_{-1} = -\sqrt{l(l+1) - r(r-1)}\,(L_1 - iL_2) \quad (17b)$$

$$\frac{2}{h^2} G'_1 = \sqrt{l(l+1) - r(r+1)}\,(L_1 + iL_2)$$

$$\frac{2}{h^2} G'_0 = 2irL_3 + \frac{t_1 + t_2}{(t_1 - t_2)^2} r^2 + \frac{1}{2}(l(l+1) - r^2) \left(\frac{t_2 + t_3}{(t_2 - t_3)^2} + \frac{t_1 + t_3}{(t_1 - t_3)^2} \right)$$

and

$$\frac{2}{h^2} F'_{-1} = \sqrt{l(l+1) - r(r-1)} \left[\frac{t_2 + t_3}{(t_2 - t_3)^2} M_1 - i \frac{t_1 + t_3}{(t_1 - t_3)^2} M_2 \right]$$

$$\frac{2}{h^2} F'_1 = -\sqrt{l(l+1) - r(r+1)} \left[\frac{t_2 + t_3}{(t_2 - t_3)^2} M_1 + i \frac{t_1 + t_3}{(t_1 - t_3)^2} M_2 \right] \quad (17c)$$

$$\frac{2}{h^2} F'_0 = -2ir \frac{t_1 + t_2}{(t_1 - t_2)^2} M_3 - \sum_p \frac{t_{p+1} + t_{p+2}}{(t_{p+1} - t_{p+2})^2} M_p^2 + 2 \sum_p L_p M_p + \frac{1}{M} \sum_{nn'} \sum_p \frac{\partial^2}{\partial y_{np}\, \partial y_{n'p}}.$$

This is the Schrödinger equation after the elimination of the six integrals of motion. The unknown functions, X_r, in (16a) may be interpreted in two ways. First, if they are considered as functions of such variables, y_{np}, which satisfy the identities (12), it can be shown that the X_r need to be defined only in the quasi-hyperboloidal regions satisfying the identities.[4] Second, they may be regarded as functions of unrestricted y's, but they must be of such a form that they are invariant under all translations and rotations. In the second case, however, the t_p must be considered as the characteristic values of the T_{ik} formed from the y's (not from the x's) and this would make the actual calculations impractical since the characteristic values of a three rowed matrix are not easily

obtainable. The terms with the F in (17) can be omitted for both interpretations, if the X_r are expressed explicitly as such functions of the y, which depend only on the relative configuration.

It will be noticed that whenever any two of the t_p become equal, the principal axes of inertia as well as the standard position become indetermined. This manifests itself in the equations by producing singularities of the first and second order for these configurations. As a result, the wave functions will not be continuous as t_p and $t_{p'}$ approach equality from the two sides $t_p > t_{p'}$ and $t_{p'} > t_p$. However, a detailed discussion of this behavior will not be given in this paper.

[1] For application to the He-atom see G. Breit, *Phys. Rev.*, **35**, 569 (1930). In a paper which appeared a short time ago (*Phys. Rev.*, **46**, 383 (1934)) C. Eckart developed the theory of rotation and inner motion of a system of particles in classical dynamics. His results are such, however, that one can deduce from his eq. (26) a quantum mechanical equation, which must be equivalent to our (17), except for the use of different coördinates. It does not seem to be quite easy, however, to specify Eckart's coördinates in such a way as to conserve the symmetry between identical particles. The elimination of the translational coördinates and applications of the resulting equation were given already by D. S. Hughes and C. Eckart, *Phys. Rev.*, **36**, 694 (1930).

[2] The system must be considered as being in a finite box in order to make the integrals finite. However, the box may be made sufficiently large so as to have no effect on the system.

[3] See any book on the applications of group theory to quantum mechanics, e.g., H. Weyl, *Theory of Groups and Quantum Mechanics*, or E. Wigner, *Gruppentheorie*, etc. Formulas (3) and the expressions for the $D^{(l)}(R)_{\mu\nu}$ may be found in the latter one in chapters XIX and XV, respectively.

[4] This can be proved directly by substituting $f(I_1, \ldots, I_6)X_r$ for the X_r in (17), where $I_1, \ldots, I_6$ are the expressions on the left side of the identities. The new X's will still satisfy (17) for all points $I_1 = \ldots = I_6 = 0$.

PLESIOMIACIS, A NEW CREODONT FROM THE SESPE UPPER EOCENE, CALIFORNIA

BY CHESTER STOCK

BALCH GRADUATE SCHOOL OF THE GEOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated December 24, 1934

Introduction.—The creodont described in this paper was found recently at Calif. Inst. Tech. Vert. Pale. Locality 207 in the Brea Canyon section of the Sespe deposits north of the Simi Valley, California. The faunal stage occurring here has a stratigraphic position approximately 675 feet lower in the section than Locality 150, and is unquestionably more closely related in age to that found at Locality 180 approximately 3 miles to the

east along the strike of the Sespe deposits. These lower faunal horizons in the Sespe are now regarded as belonging to the Upper Eocene, while the stage recorded at Locality 150 has been assigned to the uppermost Eocene.

It may be recalled that two members of the Miacidæ were described recently[1] from Locality 180. The present type is quite different from these forms and appears to be a representative of the viverravine division of the miacid family.

Viverravus (Plesiomiacis) progressus, n. subgen. and n. sp.

Type specimen.—A right ramus of the mandible with $P\overline{1}$-$M\overline{2}$, No. 1776, Calif. Inst. Tech. Vert. Pale. Coll., plate 1, figures 1, 1*a*.

Paratype.—A fragment of the right maxillary with $P\underline{4}$-$M\underline{3}$, No. 1777, plate 1, figure 2.

Locality.—Sespe Upper Eocene, north of the Simi Valley, Ventura County, California; Locality 207 C.I.T.

Subgeneric and Specific Characters.—Molars $\frac{2}{2}$. $P\overline{1}$ with single root. Anterior end of crown in $P\overline{2}$, $P\overline{3}$ and $P\overline{4}$ turned inward more than in *Viverravus sicarius*. Trigonid wider and talonid shorter in $M\overline{2}$, relative to length of tooth, than in *Viverravus*. Parastyle in $P\underline{4}$ compressed antero-posteriorly and not so well developed as in Bridger genus. Upper molars with antero-external corner extended; parastyle less distinct from paracone than in *Viverravus*. Size slightly larger than *V. sicarius*, but lower premolars shorter than in this species.

Description.—Plesiomiacis resembles the Bridger *Viverravus* in absence of the third molars, in presence of a well developed tubercle posterior to the principal cusp in $P\overline{3}$ and $P\overline{4}$, and in the characters displayed by the lower molars (except that the talonid in $M\overline{2}$ is not extended so far posteriorly). The Sespe type is more progressive than the Bridger form in reduction of parastyle in $P\underline{4}$ and in presence of a single-rooted $P\overline{1}$.

In the right ramus No. 1776, figures 1, 1*a*, the anterior premolars ($P\overline{1}$, $P\overline{2}$ and $P\overline{3}$) are more openly spaced than in *V. sicarius*. $P\overline{4}$ is shorter and relatively slightly wider than in the latter species as represented by the type specimen, No. 11521 A.M.N.H. Moreover, the trigonid is wider and the talonid is shorter in $M\overline{2}$, relative to the length of this tooth, than in No. 11521. A distinct cingulum extends along the base of the antero-external face of the paraconid-protoconid blade. In contrast to $M\overline{1}$ and $M\overline{2}$, comprising the type specimen of *Miacis* (?) *hookwayi* from the Sespe (Locality 180), the molars in *Plesiomiacis progressus* possess higher trigonids and smaller talonids. $M\overline{2}$, in particular, exhibits decidedly greater reduction in the heel region and the length of this tooth, in comparison to the length of $M\overline{1}$, is less than that in the specimen from Locality 180. The broad

posterior border of this tooth in $M(?)$. *hookwayi* may be interpreted as implying the presence of a third lower molar. Characters seen in the lower jaw and lower dentition of *Tapocyon occidentalis*, readily distinguish this type from *Plesiomiacis*.

As in *Viverravus sicarius* the anterior mental foramen in the ramus No. 1776 is situated below $P\overline{1}$ while the posterior one is situated below $P\overline{3}$. Fortunately the coronoid process is preserved in the Sespe specimen. Slightly more space prevails between the anterior border of the process and

PLATE 1

Viverravus (*Plesiomiacis*) *progressus*, n. subgen. and n. sp.

Figures 1, 1*a*, type specimen, right ramus of mandible with $P\overline{1}-M\overline{2}$, No. 1776; lateral and occlusal views. Figure 2, paratype, fragment of maxillary with $P\underline{4}-M\underline{2}$, No. 1777; occlusal view. All figures $\times 1^1/_2$.

California Institute of Technology Collections. Sespe Upper Eocene, California.

the posterior end of $M\overline{2}$ than in *V. sicarius*. The condyle is placed well above the level of the tooth-row.

In the maxillary fragment, No. 1777 C.I.T., figure 2, $P\underline{4}$ differs from the comparable tooth in the viverravine creodonts in the reduction in size of the parastyle. This cuspule is compressed and consequently the protocone projects farther forward, with reference to the anterior border of the parastyle, in *Plesiomiacis* than in *V. sicarius*. The upper molars are unfortunately considerably worn. However, it is apparent that the paracone was a distinctly larger cusp than the metacone and that the parastyle, at least in $M\underline{1}$, was extended in an outer anterior direction and was more ridge-like in its development than in *Viverravus*. The roof of the palate between

$P\underline{4}$ and $M\underline{1}$ is deeply excavated to accommodate the trigonid of $M\overline{1}$ and a similar but smaller excavation is present between $M\underline{1}$ and $M\underline{2}$, marking the position in occlusion of the trigonid of $M\overline{2}$. The space between the opposing walls of the first and second upper molars is also greater in No. 1777 than in *Miacis*.

Remarks.—Plesiomiacis from the Sespe (Locality 207) is referred to the viverravine division of the Miacidæ because of the important structural characters exhibited by Nos. 1776 and 1777, as indicated above. In reduction in size of parastyle in $P\underline{4}$ and in character of heel in $M\overline{2}$ our type from the Sespe may show greater resemblance to members of the Miacinæ. In either case, *Plesiomiacis* has advanced beyond the Bridger representatives of the Miacidæ and points toward a post-Bridger age for this stage in the history of the family.

MEASUREMENTS (IN MILLIMETERS)

	Type No. 1776 C.I.T.
Length from anterior end of symphysis to posterior end of condyle (approximate)	71.5
Depth of ramus taken between $P\overline{3}$ and $P\overline{4}$ and normal to inferior border	10.8
Depth of ramus at posterior end of $M\overline{2}$ and normal to inferior border	11.1
Length of tooth-row, $P\overline{1} - M\overline{2}$	38.3
$P\overline{1}$, anteroposterior diameter	2.6
$P\overline{2}$, anteroposterior diameter	4.8
$P\overline{3}$, anteroposterior diameter	6.4
$P\overline{4}$, anteroposterior diameter	7.3
$P\overline{4}$, greatest transverse diameter	3.0
$M\overline{1}$, anteroposterior diameter	9.0
$M\overline{1}$, width across metaconid-protoconid	4.6
$M\overline{1}$, width of heel	3.4
$M\overline{2}$, anteroposterior diameter	4.9
$M\overline{2}$, width across trigonid	2.9

	Paratype No. 1777 C.I.T.
Greatest length from anterior end of $P\underline{4}$ to posterior end of $M\underline{2}$	17.3
$P\underline{4}$, length from anterior end of protocone to posterior end of tooth	10.6
$P\underline{4}$, greatest transverse diameter	6.0
$M\underline{1}$, greatest transverse diameter along anterior face of tooth (approximate)	8.3
$M\underline{2}$, greatest transverse diameter	4.9
$M\underline{2}$, anteroposterior diameter	2.4

[1] Stock, C., *Proc. Nat. Acad. Sci.*, **20**, 423–427, pl. 1 (1934).

PROTOPLASMIC POTENTIALS IN HALICYSTIS. V. THE REVERSAL OF POTENTIAL BY UNBALANCED NACL.

BY L. R. BLINKS, R. D. RHODES AND G. A. MCCALLUM

JACQUES LOEB LABORATORY, HOPKINS MARINE STATION, AND THE SCHOOL OF BIOLOGICAL SCIENCES, STANFORD UNIVERSITY

Communicated December 31, 1934

Previously published curves[1] have showed the effect of substituting various single salt solutions for the balanced sea water normally bathing the cells of *Halicystis Osterhoutii*. There was usually a rapid disappearance of the potential difference measurable across the protoplasm, with recovery of the normal positive values if sea water was restored before too long an exposure to the unbalanced solution. Occasionally a small, transient, reversed potential preceded the drop to zero, especially with KCl treatment; this is possibly due to the superimposed special effect of K ion.[2]

A reversal of P.D to much greater negative values and for longer periods has now been produced by unbalanced, isotonic NaCl in the California species (*H. ovalis*) often enough to appear significant. Figure 1 indicates the variety of effects, ranging from only a partial loss of positive P.D., to a small and transient, or large and prolonged negative P.D. Continued exposure to NaCl always results in eventual loss of P.D.; but restoration of sea water may still cause recovery of positive values, often after a lag at zero.

Such reversal of P.D. suggests in some ways that produced by ammonia,[3] by vacuolar perfusion,[4] and by current flow,[5] although there are differences in the time curves. On the basis of such previous treatments, the NaCl effect might be explained in various ways, as follows:

(1) The outer surface might be made more permeable by NaCl so that H ions left or OH ions entered more readily from the sea water. The internal protoplasmic reaction might thus become sufficiently alkaline to lie beyond the iso-electric point of the amphoteric substances concerned in the generation of the potential (e.g., as membranes or diffusing ions), so reversing its sign. This has the theoretical objection that the P.D. reversal is too sharply critical to be represented by the smooth dissociation curves of most ampholytes. In the case of NaCl treatment, the explanation is further vitiated by the persistence of the effects over a wide pH range (from pH 5 to 9, or from the sap value to well over the sea water value).

(2) Part of the surface might be so altered that the bio-electric potential could discharge itself in local circuits (returning through holes or pores in the protoplasm). If the current density of this discharge outward across the protoplasm became sufficiently great, the P.D. would reverse, as found

by experiments with controlled current flow.[5] While it is difficult to conceive how the P.D. could remain reversed (since it would now produce a current inward, which tends to restore positivity), there are nevertheless very striking threshold and hysteretic effects, which tend to maintain a P.D., when once established, in either direction.

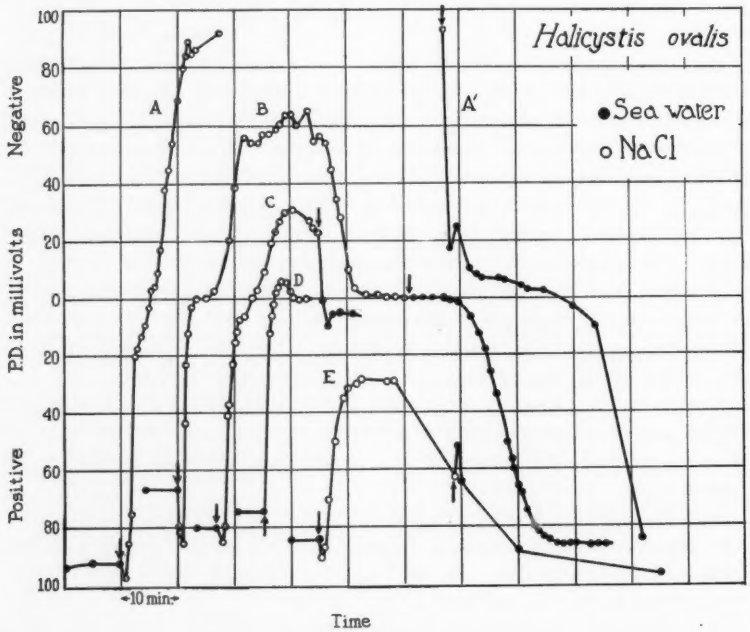


FIGURE 1

Potential difference across the protoplasm of impaled cells of *Halicystis ovalis*, as affected by exposure of the cells to unbalanced solution. Black circles represent exposure to sea water, open circles to 0.55 *M* NaCl; change of solution is indicated by arrows. Curve *A* is opened up between exposure to NaCl and restoration of sea water, and continued as *A'*, to accommodate the other curves without overlapping. Curves *A* to *E* are representative of the variety of effects found on different exposures in different cells. The sign of the P.D. is that of the external solution; when positive, the positive current tends to flow outward across the protoplasm.

(3) A much more reasonable explanation is at hand in the hypothesis already advanced by Osterhout,[6] and by Damon[7] (for *Nitella* and *Valonia*) that there are discrete potentials at each surface of the protoplasmic film, whose algebraic sum accounts for the observed P.D. across the entire protoplasm. The outer surface would presumably be first affected by unbalanced solutions, with reduction or loss of its potential, leaving the

vacuolar surface for some time unaffected, and with its potential more or less completely manifest. On this basis, minimum values of 160 mv., outwardly directed, and 80 to 90 mv., inwardly directed, might be assigned to the outer and vacuolar surfaces, respectively, to account for the observed P.D. and its reversal. The eventual loss of all P.D. could be attributed to alteration of the vacuolar surface, by penetration of the unbalanced solution to it, or to general structural or metabolic changes produced by the unbalanced solution. It is planned to test this by perfusion of the vacuole with unbalanced NaCl.

While this scheme is purely formal, and ad hoc, and explains neither the origin of the individual surface potentials, nor the manner in which they are affected by the unbalanced solution, these results give perhaps the best evidence so far adduced for the existence and considerable independence of such potentials. The two general levels, positive and negative, and the essential all or none nature of these is further so closely paralleled in the results of very different treatments, that some such arrangement or mechanism is indicated.

[1] Blinks, L. R., *J. Gen. Physiol.*, **13**, 223 (1929–30).

[2] Cf. Blinks, L. R., *Ibid.*, **16**, 147 (1932–33).

[3] Blinks, L. R., *Ibid.*, **17**, 109 (1933–34).

[4] Blinks, L. R., *Ibid.*, **18**, (1934–35) in press.

[5] Blinks, L. R., abstr. in Sunti delle communicazione, *VII Int. Physiol. Congr.* Rome (1932). A complete report on the effects of current flow is in preparation.

[6] Osterhout, W. J. V., Damon, E. B., and Jacques, A. G., *J. Gen. Physiol.* **11**, 193 (1927–28); Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.* **11**, 391 (1927–28).

[7] Damon, E. B., *J. Gen. Physiol.*, **13**, 207 (1929–30); **15**, 525 (1931–32).

HOW DO ELECTROLYTES ENTER THE CELL?

BY W. J. V. OSTERHOUT

LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Communicated December 17, 1934

Some facts which may be helpful in this connection were found in experiments on the marine alga *Valonia*.[1]

Experiments with ammonia[2] are very instructive. In the first place it is evident that ammonia does not go in chiefly as NH_4Cl for the rate of entrance falls off when we simultaneously increase the external concentration[3] of NH_4Cl and decrease that of NH_3.[4] Furthermore when the concentration of $NH_3 + NH_4^+$ in the cell is rapidly increasing very little change in Cl^- occurs.

In the second place it cannot enter chiefly by ionic exchange (as NH_4^+, in exchange for H^+ or K^+ coming out) since in that case the rate of en-

trance would be approximately proportional[5] to the external concentration of NH_4^+ but (as just stated) when this concentration increases and that of NH_3 decreases the rate of entrance falls off.

TABLE 1

pH	MOLAR* CONC. $NH_3 \times 10^4$	MOLES ($NH_3 + NH_4$) ENTERING 1 LITER OF SAP IN 10 MIN. $\times 10^4$	pH	MOLAR* CONC. $NH_3 \times 10^4$	MOLES ($NH_3 + NH_4$) ENTERING 1 LITER OF SAP IN 10 MIN. $\times 10^4$
6.10	0.0216	1.4	9.05	16.30	24.0
6.50	0.0550	3.2	9.25	23.60	27.8
6.90	0.1380	4.5	9.40	30.40	32.7
7.80	1.100	8.5	9.45	32.90	34.0
8.00	1.700	10.3	9.60	40.90	35.5
8.10	2.100	11.5	9.70	46.50	39.0
8.30	3.400	10.3	9.90	58.00	39.2
8.60	6.500	15.0			

* pK_{ab} is taken[7] as 9.76.

We may therefore conclude that it enters chiefly as NH_3. But if it diffused in as such we should expect the rate of entrance to be approximately[6] proportional to $NH_{3o} - NH_{3i}$ (where the subscripts o and i refer to concentrations outside and inside) which is not the case[7] (Fig. 1 and Table 1). How is this to be accounted for?

A very simple explanation (suggested by the study of models[8]) is that electrolytes enter by combining reversibly with a constituent HX of the protoplasm. To illustrate we may write $NH_4OH + HX \rightleftharpoons NH_4X + H_2O$. As the reaction is reversible the concentration of NH_4X at the outer surface of the protoplasm[9] and consequently the rate of entrance[10] will increase more and more slowly as the external concentration of NH_4OH increases, giving a curve of the form shown in figure 1.

The form of the curve may be ascertained by means of the formula

$$(NH_{4o})(OH_o)(HX_b - NH_4X_e) = k_1(NH_4X_e)$$

where k_1 is a constant and the subscripts b and e refer to molar concentrations at the beginning and when the reaction has reached equilibrium, respectively.[11] In this formula H_2O does not appear because its concentration is constant both in the aqueous phase and in the non-aqueous protoplasmic surface which is saturated with water.

Since $(NH_4)(OH) = K(NH_3)$, where K is the ionization constant, we may write

$$(NH_{3o})(HX_b - NH_4X_e) = k(NH_4X_e).$$

The curve shown in figure 1 is obtained by putting $k = 0.001533$ and $HX_b = 0.005$ M.[12]

The points do not fall closely on the curve: this is not surprising in view of the experimental errors and of the complications in the process of pene-

tration.[13] As the amounts entering were small the determinations were not very accurate (a few readings obviously in error were omitted).

The simplest assumption is that NH_3 enters the non-aqueous layer and reacts with HX so that the concentration of NH_3 to be used in the equation is that of the non-aqueous layer, but if the partition coefficient of NH_3 is

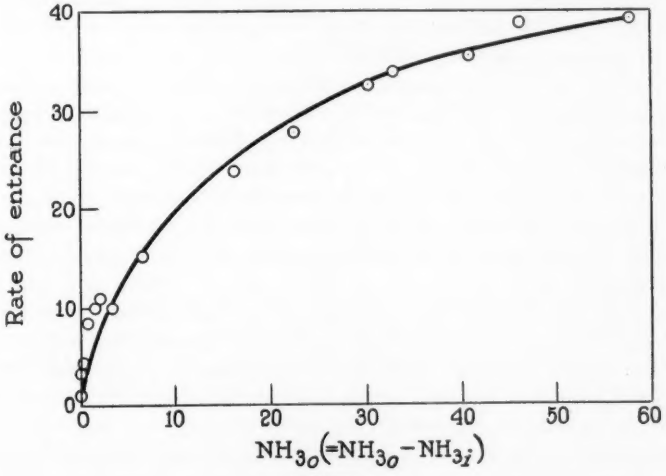


FIGURE 1

The rates of entrance (moles of $NH_3 + NH_4$ entering 1 liter of cells in 10 minutes) are plotted as ordinates and the values of $NH_{3o} = NH_{3o} - NH_{3i}$ as abscissae, where the subscripts o and i refer to concentrations outside and inside, respectively: circles show observed values.

It is assumed that NH_3 enters by combining with a constituent of the protoplasm, HX, to form NH_4X, and that the rate of entrance is directly proportional to the concentration gradient of NH_4X. The rate of entrance is taken as proportional to the concentration of NH_4X at the outer surface since in such brief experiments its concentration at the inner surface is negligible.

The curve shows the rate of entrance calculated on the basis of the reaction $NH_3 + HX \rightleftharpoons NH_4X$ from the formula $(NH_3)(HX_b - NH_4X_e) = 0.001533\ (NH_4X_e)$ where the subscripts b and e refer to the beginning of the reaction and to equilibrium, respectively (HX_b is taken as 0.005 M).

fairly constant (as is presumably the case) it will make no difference in the form of the curve whether we use the concentration of NH_3 in the aqueous or in the non-aqueous phase.[14] It seems probable that HX and NH_4X are practically insoluble in water.

Assuming that under the conditions of these experiments the rate of entrance is proportional to NH_4X at the outer surface of the protoplasm (which we may call NH_4X_o) we may write as an approximation

$$\text{Rate} = k_2 NH_4 X_o.$$

In calculating the curve in figure 1 it has been assumed for convenience that $k_2 = 1$. If k_2 really had this value the fact that HX_b is taken as 0.005 M would be significent but as we do not know the value of k_2 the value 0.005 M is of interest only for comparison with experiments on other substances. It is quite possible that HX is not the sole constituent of the non-aqueous layer: it may be merely dissolved in it.

The rise of pH in the sap caused by the entrance of NH_3 might cause the curve to flatten out somewhat by increasing NH_{3i}, but it could not explain the amount of flattening actually observed. For example, at pH 6.9 in 10 minutes the internal concentration of $NH_3 + NH_4^+$ rose by 0.00045 M which did not suffice to make any detectable rise in the pH of the sap, but the total amount entering was only about half of what would be expected judging from the first two points on the curve and considering the curve to be a straight line.

We may ask whether this could arise from the fact that in the vacuole just inside the inner protoplasmic surface the concentration and pH was greater than the figures given for the sap as a whole. But as such a factor would affect all the reported values in the same sense it might have little effect on the shape of the curve.

It might be thought that the permeability decreases as the external concentration of NH_3 increases but the experiments indicate that this is not the case.[15]

Adsorption at the surface might give a curve somewhat like that[16] in figure 1 if adsorption occurred on a micelle at the outer surface and if the micelle moved across the outer non-aqueous protoplasmic surface layer and reacted in the aqueous layer of the protoplasm[17] to form a salt which then passed through this layer, and repeated the process at the inner non-aqueous layer.

It may be added that the curve shown in figure 1 could not be produced in absence of chemical combination by the combined entrance of NH_3 entering as such by diffusion and NH_4^+ entering by ionic exchange. The rate of entrance of NH_3 would be equal to $P_{NH_3}(NH_{3o} - NH_{3i})$, where P is the permeability to NH_3, defined as the amount entering under standard conditions[18] when $NH_{3o} - NH_{3i} = 1$. The rate of entrance of NH_4^+ passing in by ionic exchange would be equal to $P_{NH_4} = (NH_{4o})(H_i) - (NH_{4i})(H_o)$, the definition of P_{NH_4} being similar to that given for P_{NH_3}. If the ratio $P_{NH_3} \div P_{NH_4}$ is constant and H_o and H_i vary but little the rate of entrance of $NH_3 + NH_4$ plotted (as in Fig. 1) against NH_{3o} will give a straight line provided NH_{3i} and NH_{4i} remain relatively small owing to the shortness of the exposure. This line will not pass through the origin and its intercept on the vertical axis will give the value of the ratio[19] $P_{NH_4} \div P_{NH_3}$.

As explained elsewhere[20] the entrance of NH_4^+ accompanied by OH^- cannot be distinguished on kinetic grounds from that of NH_3. The entrance of NH_4^+ accompanied by other anions presents a situation which will be discussed presently: it is ruled out in the present case by the facts stated above.

How do strong electrolytes act? The following possibilities may be considered.

1. Absorption takes place chiefly by ionic exchange, e g., a cation M^+ enters in exchange for H^+ coming out. The rate of entrance is then proportional[21] to $M_oH_i - M_i H_o$. When the exposure is so short that relatively little change occurs in M_i, H_i and H_o, the rate of entrance plotted against M_o should give a straight line. Experiments[22] on *Valonia* and on *Nitella* indicate that ionic exchange plays an unimportant rôle.

2. Both ions enter the non-aqueous layer simultaneously without chemical reaction. If the entering electrolyte is MOH the rate of entrance[23] will depend on the partition coefficient of MOH between the external solution and the non-aqueous layer for this will determine the concentration gradient of MOH across this layer. The rate will therefore be proportional[24] to $(M_o)(OH_o) - (M_i)(OH_i)$. Hence if we plot rate of entrance against $(M_o)(OH_o) - (M_i)(OH_i)$ we should get a straight line. When the time of exposure is short so that M_i and OH_i show relatively little change we should get a straight line by plotting rate against $(M_o)(OH_o)$.

3. The entering electrolyte reacts with a constituent of the protoplasm as in the case of NH_3. If the electrolyte is MOH, giving the reaction MOH $+ HX \rightleftharpoons MX + H_2O$ the case is like that of NH_4OH.

If acids give curves like that in figure 1 we may suppose that they unite with a constituent ROH of the protoplasm. If salts give similar curves we may consider three possibilities: 1. There are two protoplasmic constituents, HX and ROH, the former uniting with cations and the latter with anions. 2. There is an amphoteric substance which unites with both anions and cations. 3. There are present in the protoplasm acids, bases and ampholytes. Such questions must be left to future investigation.

The data in the literature give the impression that, if certain assumptions are made regarding "effective products," curves somewhat like that in figure 1 are obtainable with strong electrolytes. Experiments along this line are now being carried on.

In conclusion let us consider substances which enter against a gradient:[25] this necessitates an expenditure of energy (of which the cell has an abundant supply). As an example let us take the penetration of MOH entering when the product $(M)(OH)$ is greater inside than outside. One suggestion for dealing with this situation is to suppose that the process of penetration goes on as it would in the absence of any special expenditure of energy

but with a value of $(M_i)(OH_i)$ smaller than the measured value. If $(M_i)(OH_i) = 10^{-6}$ and $(M_o)(OH_o) = 10^{-8}$, but M neither enters nor leaves the cell, we may say that if no special energy were being expended the expected value of $(M_i)(OH_i)$ would be 10^{-8} (0.01) $= 10^{-6}$, i.e., equal to $(M_o)(OH_o)$, and this may be called for convenience the "effective value." This procedure may be regarded as somewhat analogous to that by which concentrations are multiplied by activity coefficients to make their values correspond to their behavior. In the present case the coefficient 0.01 may be called the "efficiency coefficient" and all values of $(M_i)(OH_i)$ may be multiplied by it. Thus if $(M_i)(OH_i) = 10^{-6}$ and the efficiency coefficient is 0.01 the effective product will be 10^{-6} (0.01) $= 10^{-4}$ and if $(M_o)(OH_o) = 10^{-6}$ we shall expect MOH to enter.

Summary.—Experiments on large multinucleate cells of the marine alga *Valonia* indicate that there is little penetration of NH_4^+ but that NH_3 or NH_4OH enters freely, apparently by combining with a constituent, HX, of the protoplasm.

It is not impossible that strong electrolytes also enter by combining with one or more constituents of the protoplasm.

[1] This is well suited to such studies because its multinucleate cells reach the size of a pigeon's egg and the sap can be obtained without contamination.

[2] For a description of the experiments see Cooper, W. C., Jr., and Osterhout, W. J. V., *J. Gen. Physiol.*, **14**, 117 (1930–31).

[3] In sea water to which enough NH_4Cl was added to make its concentration 0.01 M the concentrations of NH_4^+ and of NH_3 were regulated by changing the pH. There was no sign of injury in these experiments, which lasted only 10 minutes (footnote 2).

[4] The term NH_3 as here used includes NH_4OH.

[5] Really proportional to $(NH_{4o})(H_i) - (NH_{4i})(H_o)$ but NH_{4i} would be mostly negligible in such brief experiments as those here discussed.

[6] The relation is only approximate since there are a number of complicating factors (cf. Osterhout, W. J. V., *J. Gen. Physiol.*, **16**, 529 (1932–33).

[7] Cf. Osterhout, W. J. V., *J. Gen. Physiol.*, **16**, 529 (1932–33); *Ergebn. Physiol.*, **35**, 967 (1933). In calculating the concentration of NH_3 the value of pK_{ab} was taken as 9.76. This is in line with the recent work on CO_2 (cf. Buch, K., *J. conseil internat. l'exploration de la mer*, **8**, 309 (1933); *Conseil perm. internat. l'exploration de la mer, rap. et proc. verb. reunions*, **85**, 71 (1933); Buch, K., Harvey, H. W., Wattenberg, H., and Gripenberg, S., *Conseil perm. internat. l'exploration de la mer, rap. et proc. verb. reunions*, **79**, 1 (1932) which indicates that the ionic strength of sea water changes the pK by about 0.5. Taking the pK'_{ab} of NH_3 in dilute solutions as 9.26 and adding 0.5 we get 9.76 (it then becomes pK instead of pK′ since activities are taken into account). (If we use $pK'_{ab} = 9.26$ for Fig. 1 we can fit the curve approximately by putting $HX_b = 0.005$ and $K = 0.003$). As the exposure lasted only 10 minutes any change in pH due to photosynthesis may be neglected in calculating the concentration of NH_3.

[8] Osterhout, W. J. V., *Ergebn. Physiol.*, **35**, 992 (1933).

[9] As in previous papers it is assumed that the outer surface of the protoplasm is a non-aqueous layer and that a very thin film at the outer surface of this layer comes at once into approximate equilibrium with the external aqueous solution (cf. Osterhout, W. J. V., *J. Gen. Physiol.*, **16**, 529 (1932–33)).

It is assumed that the reaction is relatively rapid as compared to the inward diffusion of NH_4X so that the latter determines the time curve.

[10] The rate of entrance will depend on the inward diffusion of NH_4X and since in the present experiments (lasting only 10 minutes) back diffusion may be neglected the rate of diffusion across the outer non-aqueous surface layer of the protoplasm will be proportional to NH_4X at the outer surface of this layer. In longer experiments the back diffusion would have to be taken into account and the rate of entrance would be proportional to $NH_4X_o - NH_4X_i$ where o and i refer to concentrations in the outer and inner surfaces of this non-aqueous layer. (This is illustrated by models: in these only one non-aqueous layer is employed to represent the protoplasm but the principle is essentially the same as when two are employed.) After passing through this non-aqueous layer NH_4X presumably comes in contact with the aqueous layer of the protoplasm and reacts to form salts such as NH_4Cl. Since NH_4^+ is in equilibrium with NH_3 in this aqueous layer an increase in NH_4^+ means a corresponding increase in NH_3, as long as the pH remains constant. The NH_3 will then react with HX in the inner non-aqueous layer of the protoplasm to form NH_4X which will in turn react to form NH_4Cl, etc., on reaching the sap. (The sap is more acid than the sea water by about 2 pH units: its average pH is about 5.8 and that of the sea water 8.0.)

It seems quite possible that all these processes are so linked together that the rate of diffusion across the protoplasm will be proportional to the concentration gradient of NH_4X across the protoplasm.

Cf. Osterhout, W. J. V., *J. Gen. Physiol.* **16,** 529 (1932–33); *Ergebn. Physiol.*, **35,** 967 (1933); Osterhout, W. J. V., and Stanley, W. M., *J. Gen. Physiol.*, **15,** 667 (1931–32); Osterhout, W. J. V., Kamerling, S. E., and Stanley, W. M., *J. Gen. Physiol.*, **17,** 445, 469 (1933–34).

[11] The reaction is regarded as occurring at the surface of the external non-aqueous protoplasmic layer in such fashion that the value of NH_4X_o is quickly reached (the customary assumption is made that the outer surface of the non-aqueous layer is approximately in equilibrium at all times with the adjoining aqueous phase).

[12] The nature of this curve is such that it is not very easily manipulated by changing constants and in consequence the observed fit is regarded as significant.

[13] Cf. Osterhout, W. J. V., Kamerling, S. E., and Stanley, W. M., *J. Gen. Physiol.*, **17,** 445 (1933–34).

[14] But if we employ a substance with a higher partition coefficient than NH_3 the concentrations in the non-aqueous layer and consequently the concentration of the compound it forms with X will be greater so that the rate of entrance will be higher.

[15] Jacques, A. G., and Osterhout, W. J. V., *J. Gen. Physiol.*, **14,** 301 (1930–31).

[16] If we plot the logs of ordinates and abscissae of figure 1 we do not get a straight line such as the original adsorption formula demands but certain recent adsorption formulae differ from this.

[17] Osterhout, W. J. V., *Ergebn. Physiol.*, **35,** 1013 (1933).

[18] Osterhout, W. J. V., *Ibid.*, **35,** 990 (1933).

[19] If this ratio is unity the line will be horizontal.

[20] Osterhout, W. J. V., *Biol. Rev.*, **6,** 369 (1931).

[21] Osterhout, W. J. V., *J. Gen. Physiol.*, **14,** 277 (1930–31).

[22] Osterhout, W. J. V., *Ergebn. Physiol.*, **35,** 994 (1933); Jacques, A. G., and Osterhout, W. J. V., *J. Gen. Physiol.* **17,** 727 (1933–34).

[23] The electrolyte will be mostly in molecular form in the non-aqueous layer which has a low dielectric constant and hence permits little dissociation. Osterhout, W. J. V., *Ergebn. Physiol.*, **35,** 1009 (1933).

[24] Osterhout, W. J. V., *J. Gen. Physiol.*, **16,** 529 (1932–33); Osterhout, W. J. V., Kamerling, S. E., and Stanley, W. M., *J. Gen. Physiol.*, **17,** 445 (1933–34); Osterhout,

W. J. V., *Ergebn. Physiol.*, **35**, 991 (1933). The case where $(M_o)(OH_o) < (M_i)(OH_i)$ will be discussed later on.

[25] Jacques, A. G., and Osterhout, W. J. V., *Proc. Soc. Exp. Biol. and Med.*, **31**, 1121 (1933–34).

DEGENERATION OF XANTHOPHORES IN FUNDULUS MAJALIS

BY A. A. ABRAMOWITZ

MARINE BIOLOGICAL LABORATORY, WOODS HOLE, AND THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

Communicated December 21, 1934

Morphological color change in fishes has been the object of a long series of investigations, but in these studies, with few exceptions, only the melanophores were subjected to experimental tests. Little or no attention has been paid to the xanthophores chiefly because of their lack of distinctness both in the living animal and in histological preparations. It is the purpose of this paper to report some results obtained on the effect of variously colored backgrounds upon the formation and destruction of the yellow-bearing pigment-cells.

While it is well known that changes in the melanin content and in the numbers of melanophores of fishes may be experimentally produced by appropriate backgrounds, few such changes have been recorded for pigment cells other than the melanophores. Hewer ('27) reported an increase in the number of erythrophores per unit area of skin of an English flat-fish following a sojourn upon an orange background. Odiorne ('34) believed that the number of guanophores may be increased after prolonged adaptation to a white background. As regards the yellow pigment, the extensive study of the carotenoid content of four species of fish by Sumner and Fox ('33) constitutes the only work, as far as I am aware, in which positive results have been obtained. They were, however, "concerned very little with the xanthophores themselves" but extracted the yellow pigment from the bodies of many fishes which had been placed over white, black, red or yellow backgrounds for one or two months' duration, and compared these extractions photometrically. In Fundulus parvipinnis, one of the four species employed in their study, no changes in the total amount of xanthophyll were found after a period of about two months over these backgrounds. In the "greenfish," Girella nigricans, however, a long sojourn in a white aquarium was followed by an extensive reduction in the amount of xanthophyll. They conclude "that this reduction, which paralleled changes in the visible coloration of the fishes, was actually due to the optical environment to which they had been subjected, seems altogether pos-

sible," adding that "the significance of this result is somewhat obscured by unexplainable differences among the lots of this species." It seems probable, then, that other changes similar to, though less striking than the melanophoric alterations may be brought about experimentally in the pigmentary coat of fishes.

The method of determining quantitatively the changes undergone by the xanthophores per unit area, used in this study, was, with slight modifications, essentially that described by Odiorne ('33) for the degeneration of melanophores in Fundulus heteroclitus. For many reasons, this method could not be conveniently applied to the xanthophores of this fish. A more ideal experimental animal was found in the mayfish, Fundulus majalis, in which by far the most predominant chromatophore in the tail-fin is the large xanthophore. For this reason, the variations in the number of xanthophores in the caudal fin following long periods of adaptation to variously colored backgrounds could be studied with little difficulty. Furthermore, in the males of this species these chromatophores are larger and more distinct than in the females, and whenever possible, males were used in the following experiments, though the data include readings on females.

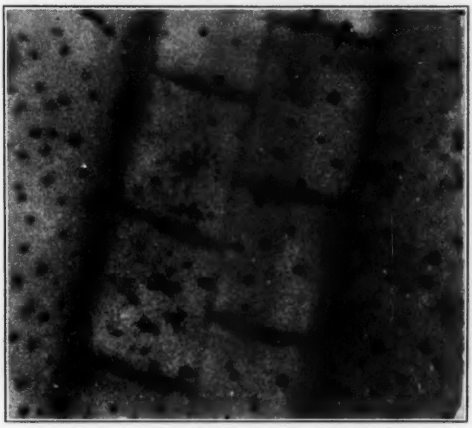


FIGURE 1

Photomicrograph (×70) of several xanthophores in the radial segments of the caudal fin of Fundulus in process of degeneration. These degenerating cells are spread out irregularly while the normal cells are punctate due to the long sojourn over a white background.

The responses of the xanthophores of Fundulus majalis parallel those described by Fries ('31) for Fundulus heteroclitus. Over a yellow or a black background, the pigment within these cells is dispersed; over white or blue backgrounds the pigment is concentrated near the centers of these cells. Such responses to backgrounds occur within an hour or so after the fishes are placed in the appropriate vessels, and are under the control of autonomic chromatophore nerves mediated through the eye.

The statement has often been repeated that a continued concentrated state of the melanophores leads to a reduction in the amount of melanin

and loss in the numbers of the melanophores; and conversely, that a continued dispersal of pigment is followed by an increase in the numbers of melanophores in the integument of fishes. In commenting upon these conclusions, and in view of his own findings, Odiorne ('34) suggested that neurohumors (Parker '32) which are responsible for the activities of chromatophores could likewise be the agents which caused quantitative melanophoric alterations. If such changes in the melanophores are produced by neurohumors, then similar changes in the xanthophores, which are probably also controlled by like substances, should be detectable.

Accordingly, about fifty fishes were distributed equally over the following four backgrounds—white, black, blue, yellow. Such backgrounds were prepared by painting the outside of large glass dishes with the appropriately colored enamel. Sea water at a temperature of about 20°C. was kept constantly running through the dishes. Since the aquarium was at some distance from the windows, it was necessary to illuminate the animals night and day throughout the entire course of the experiment with two seventy-five watt bulbs placed at a height of two feet above the vessels. Each individual fish was marked so that the same animal could easily be identified again. This species of Fundulus seldom feeds while in captivity, though attempts to feed the animal were made.

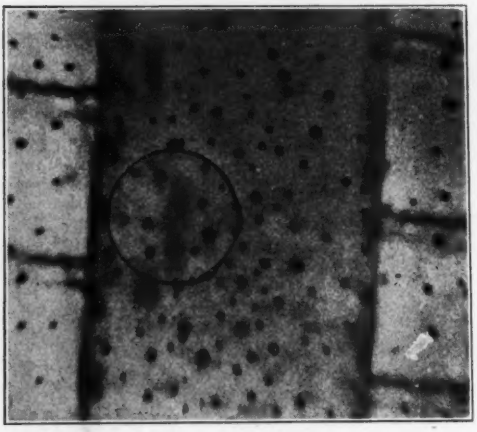


FIGURE 2

Photomicrograph (×70) of several degenerating xanthophores (encircled) in the interradial region of the tail of Fundulus. Within the circle, the degenerated cells appear as gray blotches, the normal cells as punctate bodies.

In order to obtain quantitative data, a transverse cut was made through a fin ray in the tails of all the animals at about one third the distance from the base of the tail. This served as a convenient landmark in finding the same area. The xanthophores were counted in two segments of the second fin ray ventral to the transected ray. These two segments in the second ventral ray were the ones immediately below the transverse cut. Three counts of this area were made on only the left side, and the average taken. Since there were rarely more than fifty cells in this area, the varia-

tions in the counts never exceeded two or three cells. In most cases, a free hand sketch was made so that there could be no question that the same area was counted throughout the experiment.

Due to the great mortality of Fundulus majalis while in captivity, only three fishes survived a 44 day adaptation to the backgrounds. Most of the fishes which died during the course of the experiment showed slight changes in the numbers of the xanthophores of the area. Only the results from animals which lived throughout the major part of the experiment will be given here:

A. White background (13 fishes)

The number of xanthophores was reduced from 235 to 212 in one group of five surviving animals after 17 days, a reduction of 9 per cent. One fish lived 44 days, during which time the number of cells was reduced from 52 to 26, a loss of exactly one-half the original number.

B. Yellow background (9 fishes)

There was an increase from 156 to 164 cells in a group of three fishes surviving after 17 days, a 5 per cent increase. None of these animals lived more than 20 days.

C. Blue background (11 fishes)

In three remaining animals after 13 days, there was a decrease from 122 to 112 cells, a 9 per cent decrease. One fish survived 41 days and showed a reduction from 61 to 33 xanthophores, a loss of about 55 per cent.

D. Black background (8 fishes)

A 3 per cent increase was noted in four animals living after 12 days (257 to 264 cells). One fish showed an increase from 86 (the highest individual count) to 97 xanthophores, a 12 per cent increase after 44 days.

FIGURE 3

Photomicrograph (×70) of a mass (encircled) of melanin and xanthophyll particles accumulated near the surface of the epidermis of the tail of Fundulus. The melanin is concentrated in the large, dark, central area. The carotenoid particles photograph as gray and mainly surround this central area.

These changes which take place in the relatively small experimental area of the tail are characteristic for the entire tail and probably for the body as a whole, although no attempts were made to study such changes elsewhere. Figure 2 shows several xanthophores of the interradial region in the process of degeneration; figure 1 shows the same changes in the ray

segments. These same changes are widespread throughout the entire fin.

The exact manner in which the xanthophores disintegrate has not been studied. In many respects, they seem to behave much like the degenerating melanophores which, according to Koketsu ('15) and Tozawa ('24), are largely phagocytized and sloughed off through the epidermis. In any event, the yellow pigment, upon reaching the surface of the skin, is accumulated in large masses which are then sloughed off. Interesting in this connection is the condition found in animals kept over a white background. Here, the melanophores, though considerably fewer in numbers than the xanthophores of the caudal fin, also degenerate at the same time that this process is going on in the xanthophores. Figure 3 shows a large mass (encircled) of pigment particles formed from degenerating cells. The dark central area is composed of melanin particles and is surrounded by the carotenoid particles which photograph as gray. This dark central mass, however, not entirely melanin for small fragments of yellow pigment have been observed among the predominant black masses.

While only a small number of animals survived the entire duration of the experiment, it seems, nevertheless, quite clear in these that a sojourn over blue, or white backgrounds, in which the xanthophores are in a concentrated state, is followed by a 50 per cent decrease in the number of the carotenoid cells. Over yellow, or black environments, in which the pigment is dispersed throughout the cells, there is an increase of 5 per cent (yellow for 18 days) and of 12 per cent respectively for about the same amount of time. If these results, which pertain to changes in the numbers of xanthophores, are comparable to variations in the total amount of xanthophyll extracted from the bodies of many fishes, then Fundulus majalis apparently differs from Fundulus parvipinnis and seems to resemble Girella nigricans in this respect.

Fries, E. F. B., "Color Changes in Fundulus, with Special Consideration of the Xanthophores," *Jour. Exp. Zoöl.*, **60**, 389–426 (1931).

Hewer, H. R., "Studies in the Colour Changes of Fish. Part II. An Analysis of the Colour Patterns of the Dab," *Phil. Trans. Roy. Soc., London*, **B215**, 177–200 (1927).

*Koketsu, R., "The Color Change of the Young Goldfish," *Dobutsujaku Zasshi*, **27** (Japanese) (1915).

Odiorne, J. M., "Degeneration of Melanophores in Fundulus," *Proc. Nat. Acad. Sci.*, **19**, 329–332 (1933).

Odiorne, J. M., "Experimental Alterations in the Pigmentation of Fishes," (in press) (1935).

Parker, G. H., *Humoral Agents in Nervous Activity*, Cambridge, 79 pp. (1932).

Parker, G. H., "The Expansion and Contraction of Chromatophores," *Science*, **79**, 428–429 (1934a).

Parker, G. H., "Cellular Transfer of Substances, Especially Neurohumors," *Jour. Exp. Biol.*, **11**, No. 1, 81–88 (1934b).

Sumner, F. B., and Fox, D. L., "A Study of Variations in the Amount of Yellow Pig-

ment (Xanthophyll) in Certain Fishes, and of the Possible Effects upon This of Colored Backgrounds," *Jour. Exp. Zoöl.*, **66**, 263–301 (1933).

* Tozawa, T., "Notes on Experiments on the Color Change of the Goldfish," *Dobutsugaku Zasshi*, **36**, 373–376 (Japanese) (1924).

* Cited from Odiorne '35.

REGENERATION OF CHROMATOPHORE NERVES

BY A. A. ABRAMOWITZ

BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

Communicated December 21, 1934

The purpose of this paper is to report a simple and accurate method of studying the regeneration of autonomic pigment motor fibres. If a transverse cut is made across several fin rays in the base of the tail of Fundulus heteroclitus, the killifish, a dark band is produced due to the dispersion of pigment granules within the melanophores which the interrupted nerves previously supplied (Wyman, '24, Mills '32, Parker and Porter '33). Within several days, the interrupted nerves degenerate progressively from their cut ends toward the periphery (Parker and Porter '33). Some time later, nerves will grow out from their central stumps into the denervated area, and this process may now be studied by the reactions of the melanophores as they receive the regenerated nerve fibres.

The changes in tint of Fundulus over white and black backgrounds, as indicated by the work of Mills ('32), are probably under the control of a double innervation. Stimulation of one set of fibres reflexly by a white background, or by electrical stimulation of the medulla or roof of the mouth, results in a concentration of the pigment within the melanophores, and the fish assumes a light tint. Conversely, stimulation of the opposite set by a black environment, or by nerve section brings about a dispersion of the pigment and the dark coloration of the animal. These changes in the extent of the melanin within these chromatophores are brought about within five minutes and hence they may serve as indicators of innervation during the course of regeneration.

The fate of the dark band which results from the interruption of nerves by a transverse cut across one or two fin rays in the caudal fin of this fish is dependent upon neurohumors (Mills '32, Parker '34a). While the cells within the denervated area are incapable of responding nervously to background, they can, however, be influenced by long periods of adaptation to white and black backgrounds. Over the former of these environments, this dark band pales entirely within two days so that the denervated area is no different in tint from that of the rest of the tail. Conversely, over

black surroundings, this band remains equally as dark as the tint of the animal. Utilizing the responses of melanophores to backgrounds as indicators of nervous activity, regeneration of nerves to these denervated areas, therefore, may be studied in two ways.

One method is that which has been utilized by Parker and Porter ('33). A transverse cut was made in the tails of many fishes. These were then kept in an illuminated white vessel for less than a month, during which time there resulted a concentration of the melanophores in the denervated as well as in the innervated portions of the fin. To measure the extent of regeneration, another transverse cut was made anterior to the primary cut in several animals. Since dispersion of pigment following nerve section is due to the stimulation of the dispersing fibres (Parker '34b), then the dark stretch formed in the originally denervated area is a measure of the extent to which these dispersing nerves have regenerated. Such a procedure, however, renders the fish unserviceable for further determinations since now a freshly denervated band is formed by the secondary cut. Making such cuts in different fishes on successive days, they found that the process of regeneration was completed 25 days after the initial incisions were made. The first signs of regenerative growth into the area were noted only after 18 days. Thus, 7 days were required for nerves to regenerate a distance of 6 millimeters, which was the distance from the primary cut to the posterior edge of the melanophore area of the tail. The rate was, therefore, computed to be about 0.86 mm. per day.

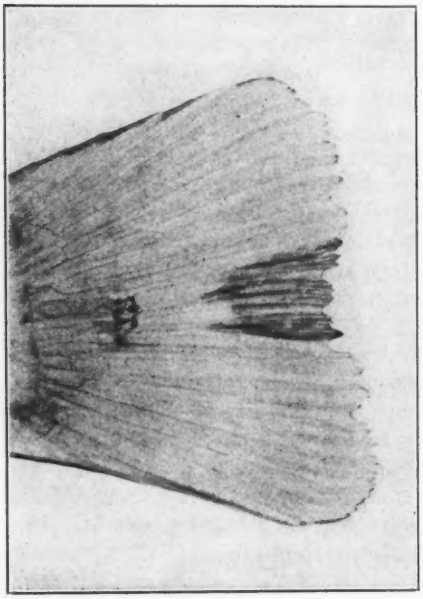


FIGURE 1

Photograph (×2) of the caudal fin of Ameiurus. The light region distal to the cut marks the region to which nerves have regenerated. The dark area of the originally denervated band has not yet received regenerated concentrating fibres.

This rate, however, may not be an entirely accurate determination. The assumption that regeneration begins approximately simultaneously in

many fishes is not altogether a true one, for it is rare that more than 20 per cent of the animals will show regenerative growth on the same day. It is thus conceivable that a slight error may have been made in the determination of this rate due to the fact that regeneration may have begun in some of the animals and not in the others at the 18th day. These authors point out that a more ideal method of study would be to measure daily on the same fish the extent of the invading nerve fibres, a method which can be realized by the following experiment.

Instead of keeping the experimental animals in the light condition, they are placed for two weeks in black walled vessels. The fishes including their denervated bands are dark. Every two days, the fishes are placed for 5 minutes in an illuminated white vessel. The fishes become pale excepting for the denervated bands which remain dark. Such a condition persists for two or three weeks after the primary cut has been made. Sometime during the second week, however, not only will the fishes become light but also a small portion of the dark band distal to the cut will lighten when the animals are placed over a white background for 5 minutes. This indicates that the denervated portion of the tail is receiving regenerating nerves, which, when appropriately stimulated, will bring about the concentrated state of the melanophores. This light area distal to the incision can easily be measured with an ocular micrometer. This procedure is repeated daily, and the length of the band which responds reflexly to the background increases in extent. The process of regeneration is complete when the originally denervated band will respond entirely to the background as does the rest of the tail.

Upon handling, Fundulus darkens rapidly in most cases, and consequently a quick method of making measurements had to be devised. Such a method is made possible by the segmentation of the fin rays. Since the transverse cut in all cases extended over two rays and the intervening interray segment, the more ventral of the cut rays could be used as a measuring rod. By counting under the binocular microscope on only one face of the fin the number of segments from the cut to the distal edge of the light, and therefore regenerated region of the interray, daily measurements could easily be made. After regeneration was completed, the fins were cut off the fishes, and the original measurements in numbers of ray segments were converted into millimeters per day by use of an ocular micrometer. As is evident from the study of many animals, the rate of regeneration of the concentrating nerve fibres may vary from day to day. Part of this variation is, no doubt, due to an error in making the readings. The average for a group of six animals was 0.55 mm. per day during the summer months. Moreover, it is no different in fishes which were starved from those which were fed during the period of regeneration.

Regeneration of the dispersing fibres may be studied in much the same

way. After the area innervated by the concentrating fibres is measured as described above, the fish is replaced on a black environment for 5 minutes. Approximately the same area which lightened over white now darkens along with the animal as a whole due to the black background. This indicates that the dispersing fibres regenerate at a rate equal to that of the concentrating fibres, and indeed this is to be expected. However, as will be reported in another communication, these two sets of nerves do not always regenerate to the same cells at the same time, and hence the area in which the melanophores respond to white environment is not, cell for cell, identical with that in which the cells respond to black.

That both sets of nerves regenerate at the same speed is made even more obvious by the following experiment. Several fishes in which signs of regeneration have become evident are placed in white vessels until the entire tail is in the light condition. These fishes are then placed over an illuminated black vessel for 5 minutes, whereupon the animals darken including that part of the region distal to the cut which has received dispersing fibres. This area of regeneration as indicated by a dark streak increases in extent daily, and the rate for this increase was found to be approximately that for the concentrating fibres.

These experiments were repeated on the catfish Ameiurus nebulosus, which in some respects proved to be a better experimental animal than Fundulus because the area in which regeneration could take place was greater. In every respect the results found for Ameiurus were similar to those already described for Fundulus. The rate for the regeneration of the concentrating fibres is about 0.5 mm. per day. Figure 1 shows the tail of a catfish which was cut on May 5th, and placed over black surroundings for several weeks. It was then placed over a white background for one hour, during which time the animal became pale including that part of the area distal to the incision which had received concentrating motor fibres. The unregenerated portion of the tail remains black. This dark region decreases in extent as regeneration proceeds, until finally, the entire area distal to the cut will lighten when regeneration is complete. These experiments were also repeated, with identical results, using electrical stimulation of the roof of the mouth instead of a white background to cause a concentration of the melanophores. The dispersing nerves also regenerate at the same rate as the concentrating fibres of this fish, and can be measured in the manner already described for Fundulus. Here again, the region innervated by one set does not always agree with that innervated by the opposite set.

The rate of regeneration of peripheral nerves as determined by this method falls between the highest and lowest rate for the same animal given by Speidel '33 (1.44 and 0.20 mm. per day) for frog nerve. In Fundulus and Ameiurus, the process of regenerative growth does not begin simul-

taneously in all animals, but when once begun, the average rate with which it proceeds is a constant one for many animals. This rate is the same for both sets of nerves which control the activities of the chromatophores.

These results were developed during the course of a problem carried out under the direction of Professor G. H. Parker. It is a pleasure to acknowledge here my gratitude for his many kindnesses and valuable suggestions during this work.

Fries, E. F. B., "Color Changes in Fundulus with Special Consideration of the Xanthophores," *Jour. Exp. Zoöl.*, **60**, 389–426 (1931).

Mills, S. M., "The Double Innervation of Fish Melanophores," *Jour. Exp. Zoöl.*, **64**, 231, 244 (1932).

Parker, G. H., "Cellular Transfer of Substances, Especially Neurohumors," *Jour. Exp. Biol.*, **11**, no. 1, 81–88 (1934a).

Parker, G. H., "Neurohumors as Activating Agents for Fish Melanophores," *Proc. Am. Phil. Soc.*, **74**, no. 3, 177–184 (1934b).

Parker, G. H., and Porter, H., "Regeneration of Chromatophore Nerves," *Jour. Exp. Zoöl.*, **66**, 303–307 (1933).

Speidel, C. C., "Studies of Living Nerve. II. Activities of Ameboid Growth Cones, Sheath Cells and Myelin Segments, as Revealed by Prolonged Observation of Individual Nerve Fibres in Frog Tadpoles," *Am. Jour. Anat.*, **52**, 1–79 (1933).

Wyman, L. C., "Blood and Nerve as Controlling Agents in the Movements of Melanophores," *Jour. Exp. Zoöl.*, **39**, 73–131 (1924).